EPIDEMIOLOGY AND PATHOGENESIS OF FASCIOLOSIS IN EASTERN NEPAL

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DECLARATION

I hereby declare that this thesis describes research carried out by myself under the supervisions of Dr. L.J.S. Harrison, Dr. J.A. Hammond and Mr. F.E. Tollervey unless otherwise cited or acknowledged. It has not, in whole, or in part, been previously presented for any other degree.

Shubh N. Mahato

This thesis is dedicated to my wife,

Shail Kumari Mahato

and my children,

Sandip, Srijana and Sanju.

CONTENTS

ABBREVIATIONS AND SYMBOLS

Ci	Curie, unit of radioactivity
$CsCl_2$	Cesium chloride
CTAB	Cetyltrimethyl-ammonium bromide
CTVM	Centre for Tropical Veterinary Medicine, Edinburgh, Scotland
°C	Degree centigrade
dATP	2'-deoxyadenosine-5'-triphosphate
dCTP	2'-deoxycytidine-5'-triphosphate
dGTP	2'-deoxyguanosine-5'-triphosphate
DNA	Deoxyribonucleic acid
dpm	Disintegration per minute
dTTP	Thymidine-5'-triphosphate
EDTA	Ethylenediamine tetra-acetic acid
EPG	Eggs per gram of faeces
g	Relative centrifugal force
g	Gram, unit of weight
GGT	γ-glutamyl transpeptidase
GLDH	Glutamate dehydrogenase
H_2O	Water
HCl	Hydrochloric acid
IPTG	Isopropyl-β-D-thiogalactopyranoside
kb	Kilo base pairs
kg	Kilogram
LB	Luria-Bertani
M	Molar
mA	Milliampere
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
mg	Milligram
$MgSO_4$	Magnesium sulphate
min	minute
ml	Millilitre
mm	Millimetre
mM	Millimolar
μCi	Microcurie
μg	Microgram
μl	Microlitre
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng	Nanogram
OD	Optical density
^{32}P	A radioactive isotope of phosphorus
P	Probability

PAC	Pakhribas Agricultural Centre, Dhankuta, Nepal
PBS	Phosphate buffered saline
PCV	Packed cell volume
pfu	Plaque forming unit
RBC	Red blood cells
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulphate
SE	Standard error of mean
SM	Sodium chloride/magnesium sulphate
SSC	Sodium chloride/sodium citrate
TAE	Tris-acetate/EDTA
TARS	Tarahara Agricultural Research Station, Sunsari, Nepal
TE	Tris/EDTA
v/v	Volume by volume
VFS	Veterinary Field Station, Royal (Dick) School of Veterinary Studies
w/w	Weight by volume
WBC	White blood cells
WPI	Weeks post infection

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SUMMARY

Following a general introduction, the thesis is divided into three parts, covering epidemiology, pathogenesis and molecular biology. Each part contains relevant literature review, materials and methods, results and a discussion for that particular part. The thesis ends with a general discussion which, includes an estimate of the financial loss resulting from fasciolosis in Nepal but concentrates on the major conclusions reached from this study and the major recommendations.

In part one, following a review of literature on the factors affecting epidemiology of fasciolosis, a 19 months field survey on the epidemiology of fasciolosis in eastern Nepal is described. Four *Lymnaea* spp., namely *L. auricularia* race *rufescens*, *L. auricularia sensu stricto, L. viridis* and *L. luteola* were identified. *L. auricularia race rufescens* was the predominant species. The main snail habitats consisted of springs or stream fed rice-fields, irrigation channels, ponds and road-side pools. The monsoon rains and rice cultivation practices contributed to the creation and expansion of the snail habitats. The snail population density was high during the dry period and declined with the onset of the monsoon. Snail egg masses and young snails were observed throughout the year. In the hills, mature *Fasciola* spp. infections were found in snails from May to February, while in the Terai, infected snails were found throughout the year.

In the hills, the highest prevalence of fasciolosis was recorded in buffaloes (57.9%) followed by cattle (44.8%), goats (22.4%) and sheep (18.2%). The relatively high prevalence of fasciolosis observed in stall-fed buffaloes was due to feeding metacercariae contaminated, rice-straw and grass, cut and carried from rice-fields. In the Terai, the highest prevalence was found in cattle (51.4%) followed by buffaloes (41.3%) and goats (13.3%). Slaughter place surveys revealed that *F. gigantica* is the predominant species, although infection with *F. hepatica* and an intermediate form were also found. The mean fluke burden in infected buffaloes was 203.2 $\pm$ 17.9, in infected goats this was 23.7 $\pm$ 3.3. Fluke burdens increases with the age of the animals. The seasonal pattern of the infections in the intermediate and definitive hosts indicated that the bulk of the infection was derived from fluke eggs deposited on the

pasture during March-May and again in October and November.

A control programme for fasciolosis based on these findings requires that all animals, including goats and sheep, be treated with appropriate anthelmintics, such as oxyclosanide or triclabendazole, in February and again in late August.

In part two, the literature on the pathogenesis of fasciolosis with special reference to its effects on productivity of ruminants is reviewed. Descriptions of the 5 experimental studies on the pathogenesis of fasciolosis carried out in Edinburgh and in Nepal follow. A pilot comparative study in sheep, conducted in Edinburgh indicated that *F. gigantica* was more pathogenic than *F. hepatica.* A second pilot experiment was conducted in Nepal using 11 to 14 months old 9 Baruwal sheep. With a mean burden of only 5 *F. gigantica*, the mean weekly liveweight gain of the infected sheep over 35 weeks was 22.0% less than that of the uninfected controls. The pathogenesis in goats was investigated using 15 to 18 months old 18 Nepalese hill goats. Burdens of more than 1.3 flukes/kg of initial liveweight produced clinical chronic fasciolosis. A burden of 3.6 flukes/kg caused death of a goat at about 33 weeks after infection. There was a significant reduction in weight gain in the infected goats.

Two experiments were carried out to study the pathogenesis of fasciolosis in 12 to 18 months old 32 Nepalese hill buffaloes. During the 35 weeks of post infection monitoring, no clinical signs were observed in the buffalo calves which harboured $\leq$ 1.0 fluke/kg of initial liveweight, while $\geq$ 1.3 flukes/kg resulted in clinical fasciolosis. A chronic disease occurred in those 6 buffalo calves which had burdens between 1.3 and 4.9 flukes/kg. Two of these became moribund around 26 weeks after infection while the remaining 4 survived until the end of the experiment. The presence of 7.4 to 9.1 flukes/kg caused subacute fasciolosis resulting in the death of buffalo calves between 14 and 18 weeks after infection. In both experiments, the liveweight gain of the infected buffaloes was affected; a longer infection period with smaller fluke burden has a proportionally greater effect on the depression of liveweight gain than a shorter infection period with larger fluke burden. The mean dressing percentage was reduced by about 12.4% in the infected buffaloes.

Although, eosinophilia and the increased serum GLDH and GGT levels appeared to be the sensitive indicators of *Fasciola* spp. infections, these were not related to the intensity of infections. Infiltration of eosinophils in periportal areas and bile ducts lamina propria was markedly evident in sheep and goats, but not in buffaloes. The inferior eosinophilia together with the absence of liver tissue infiltration by eosinophils in infected buffaloes may well be related to the fact that buffaloes have poorer ability to develop resistance to infection of *F. gigantica* even than sheep and goats. A prominent feature in subacute infections in sheep and buffaloes was aberrant flukes in various organs and accompanying haemorrhagic areas.

In part three, the available literature on the advantages and disadvantages of the various methods available for speciation and differentiation of *Fasciola* spp. is reviewed. This is followed by a description of the molecular biology work which was undertaken and which resulted in the development of species-specific (MHFh and MHFg) and cross-reactive (MHFx1 and MHFx2) DNA probes for the identification of *Fasciola* spp. None of the probes reacted with any of the control or host DNA's tested. If used in conjunction these DNA probes can clearly differentiate between *F. hepatica* and *F. gigantica*. As such they present useful tools for species identification.

GENERAL INTRODUCTION
AND
BACKGROUND

CHAPTER ONE

GENERAL INTRODUCTION AND BACKGROUND

1.1 The country: Nepal

Nepal is a landlocked Asian country situated between China and India. It occupies an area of 147,181 sq km and is of rectangular shape having an east-west axis of some 880 km long and a north-south axis that varies between 130 and 240 km. Some of the most diverse physiographic conditions in the world are found in Nepal. These range from subtropical alluvial plains at an altitude of 60 m to the high Himalayas stretching along the full length of the northern border with Tibet (China), with eight out the world's ten highest peaks, including Mount Everest at 8,848 m.

Physiographic and socio-economic factors are the major determinants of the composition of the ecological environment at any location in Nepal. Therefore, a brief description of these factors along with some background information of the country are presented in this chapter.

1.1.1 *Physiographic divisions*

Nepal is divided into five broad physiographic regions: (i) Terai, (11) Siwalik hills, (iii) Middle Mountains, (iv) High Mountains and (v) High Himal (Figures 1.1 and 1.2).

The ***Terai***, adjacent to India, is an almost continuous strip of alluvial deposits, never more than 50 km wide, stretching in an east-west direction along the southern side of the Siwalik ranges. The elevation ranges from 60 m near the Indian border to 330 m near the hills. The Terai has a humid sub-tropical climate with rainfall averaging 1,600 mm per year, mostly during the June-September monsoon. It accounts for 14 percent of the country's total area, 42 percent of the cultivated area, and supports 37 percent of the population (LRMP, 1986).

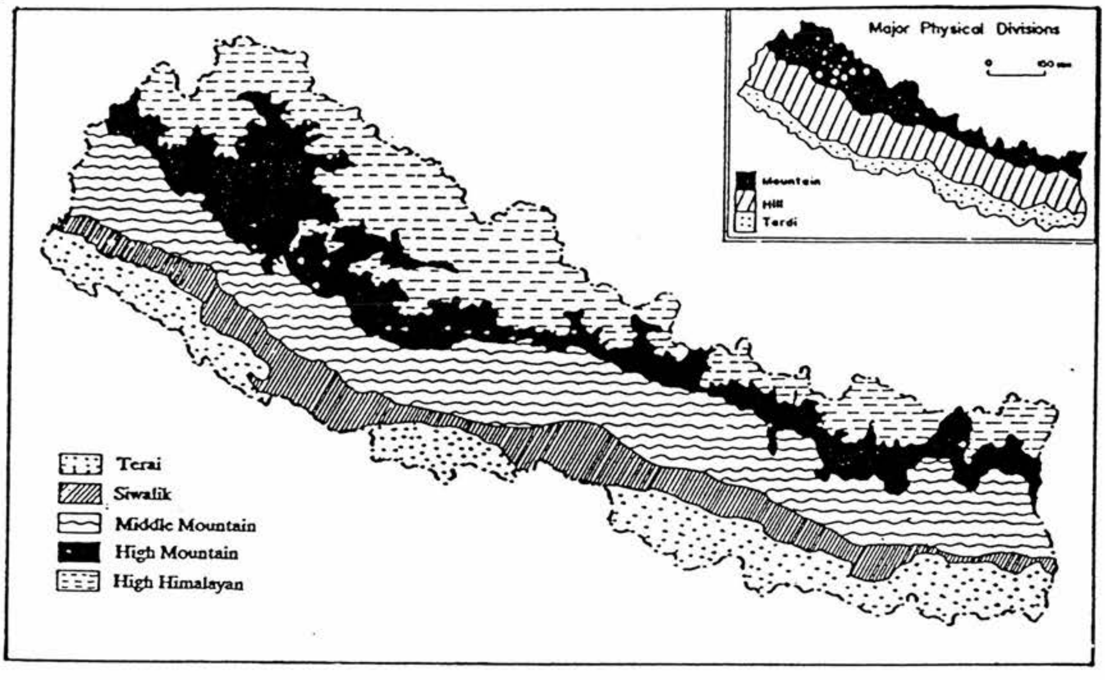


Figure 1.1. The five physiographic divisions of Nepal with an inset to show the areas covered by the commonly used terminology of mountain, hills and Terai.

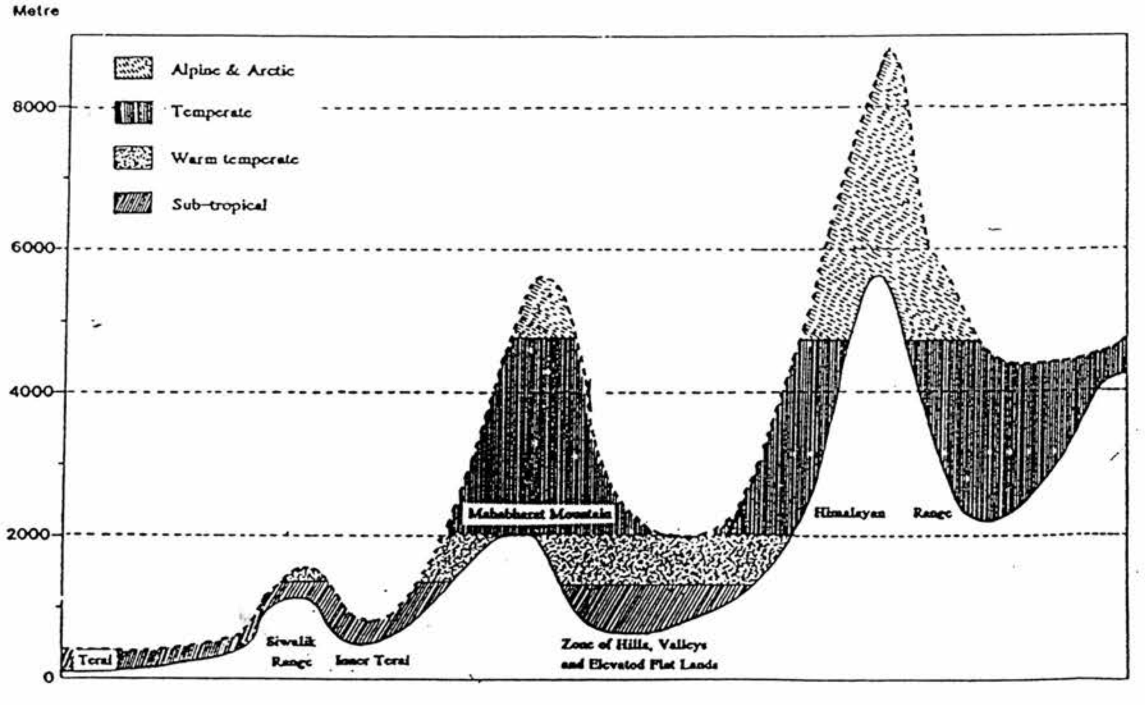


Figure 1.2. Cross section of Nepal showing climates at the different altitude ranges.

The ***Siwalik hills***, also running in a north-west to south-east belt some 800 km long, range from 200-1500 m in altitude. The climate is sub-tropical (but warm temperate at higher elevations). The high intensity rainfall occurring mostly during the June-September monsoon. The Siwaliks accounts for 15 percent of the country's total area, 9 percent of the cultivated area, and supports 8 percent of the population (LRMP, 1986).

The ***Middle Mountains*** lie north of the Siwaliks, in a belt 40-60 km wide, running the length of the country. They are made up of high ridges and steep valleys embracing numerous streams and rivers. Elevation ranges mainly from 800 to 2,400 m, but includes peaks up to 3,000 m. The climate is generally temperate with considerable variation in micro-environments, created by the rugged topography, and varying according to elevation, direction and steepness of slope, solar radiation and cloud cover. The Middle Mountains account for 29 percent of the land area, 41 percent of cultivated land, and supports 46 percent of the population.

High Mountains, which range from 2,200 to 4,000 m, has no clearly defined boundary with the Middle Mountains. Extensive areas of grazing lands are found in the form of alpine pastures. Migrating livestock from lower areas utilize the pastures of this area during the monsoon. The High Mountains accounts for 19 percent of the country's total area, 8 percent of the cultivated area, and supports 8 percent of the population (LRMP, 1986).

The ***High Himal*** belt, comprising the High Himalayas, parallels the northern border with Tibet (China) at altitudes above 4,000 m. It is mainly characterised by high peaks, steep slopes and narrow valleys. Climate is predominantly arctic, with permafrost, permanent snow fields and many glaciers. The little agricultural land available is found in the valleys and in some cases in sheltered pockets of the hill slopes. Pasture lands are used by migratory livestock which winter in the High Mountain region, and by yaks passing along trade routes connecting to Tibet. The High Himal contains 23 percent of the land area, and a very small portion of cultivated land and less than 3 percent of population.

1.1.2 *Administrative divisions*

Administratively, Nepal has five development regions, 14 zones, 75 districts, 4,000 Village Development Committees and 36,000 wards or settlements. The regional divisions (Figure 1.3) delineated for development purposes are artificial divisions for administrative convenience with no objective commonalities. The **Far Western** region with only two zones is obviously the smallest one. The **Mid Western** region is the biggest but least developed. The middle and southern parts of **Western** region are well developed. As regards to economic development, the **Central** and **Eastern** regions are far ahead of the other regions.

While the population of the Terai and few urban centres of the hills generally have good road access, the hill and mountain regions are only accessible by foot. In the hills and mountains, distances are measured in terms of the number of days it takes to cover them, and millions of people live more than a day's walk from the nearest road or air strip. Most goods are carried in and out of these regions by porters and pack animals such as yaks (*Bos peophagus*), yakows (*B. peophagus* X *B. indicus*), horses, mules, goats and sheep.

1.1.3 *The People*

In 1991, the total population was estimated to be 18.5 million with a growth rate of 2.1 percent per year for the past decade (CBS, 1993). The average population density for the country was 125.4 per sq km. The population per sq km of cultivated land averaged 472 people for the whole country, ranging from 658 person per sq km in the hills to 365 persons per sq km in the Terai (DFAMS, 1990).

Nepal has a composite population stemming from various ethnic groups. The people of the high mountain areas are mainly of Tibetan origin, with their culture, traditions and lifestyles greatly influenced by Buddhism and Tibetan culture. They have a long history of pastoralism and trading activities based on livestock. Indo-Nepalese "caste- Hindus" make up two- thirds of the population of the hills. However, many hill people are of Tibeto-Burman origins. In these ethnic groups women have a greater role in decision making than in high caste Hindu society.

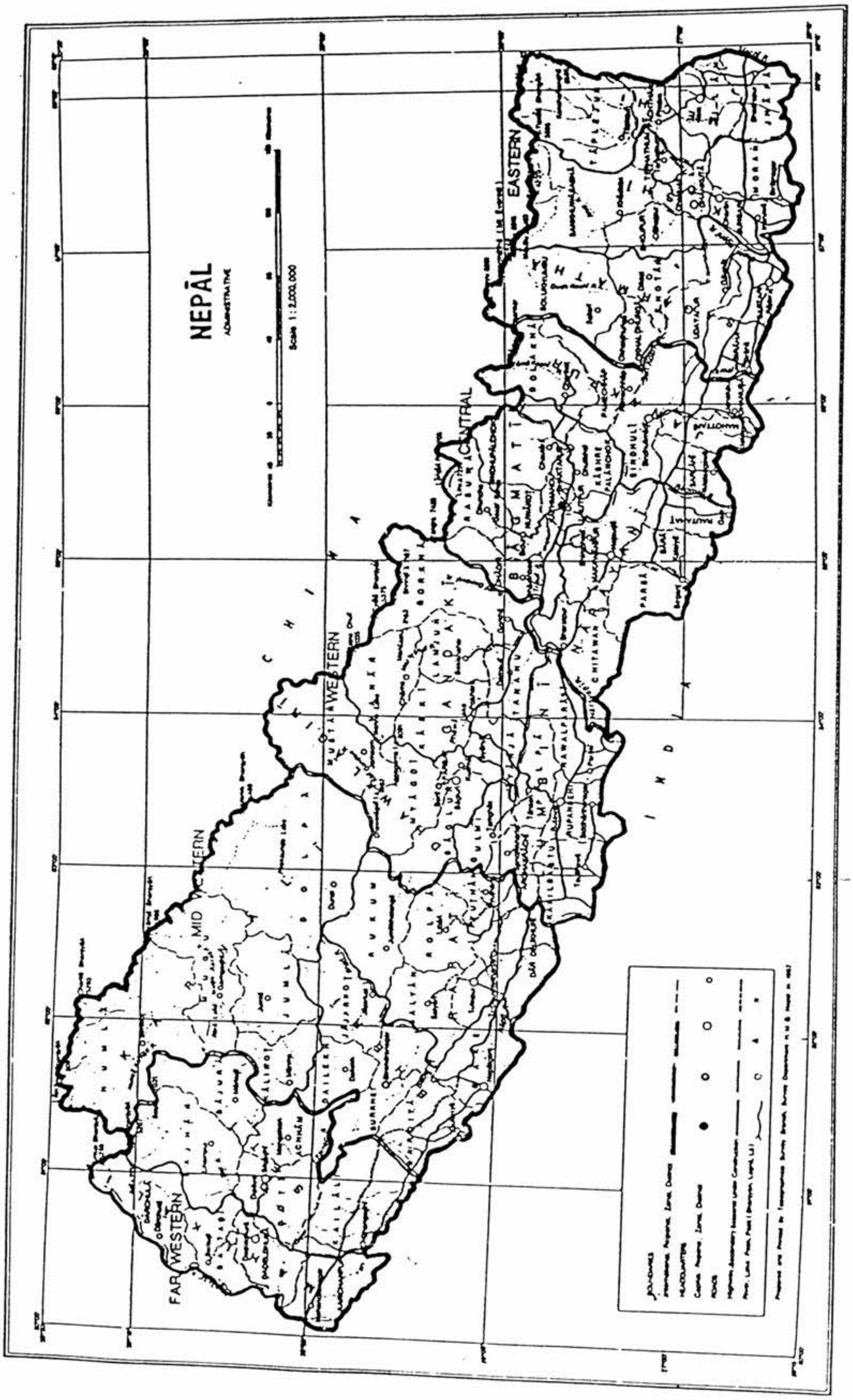


Figure 1.3. Map of Nepal showing administrative divisions at the regional and district levels.

The Terai population is comprised mainly of people of Indian origin, predominantly Hindu, but also including a significant Muslim minority and increasingly people of Tibeto-Burman origin as a result of migration from the hills. Ethnic factors affect the way in which resources are used, through attitudes towards diet, different agricultural practices, keeping of livestock, the role of women, and the allocation of property rights.

1.1.4 *Farming Systems*

Over 90 percent of the population in Nepal is classified as rural. These and many of the urban population depend largely on subsistence farming for their livelihood. The nature of farming systems employed, which influences the use of natural resources and ecological impact, is largely determined by a combination of and interactions between climate, topography, altitude, social organisation, religious beliefs and taboos, access to and ownership of certain classes of land and to markets. A wide variety of farming systems and great diversity within them exist in Nepal.

The average farm size in the country is 1.92 ha, being lower in the hills (1.12 ha) than in the Terai (2.31 ha)(DFAMS, 1990). In the hills, 43 percent of the population have holdings of less than 0.5 ha which is insufficient for the needs of an average family of 5.8 persons. Throughout the country, agriculture, livestock and forestry production systems are interdependent.

1.1.5 *Importance of Livestock*

Livestock play an important role in the farming systems and provide a major source of animal protein and household cash income, and thus have a significant place in the national economy. They comprise 18.2 percent of the agricultural gross domestic product (AGDP), and the AGDP accounts for 58.2 percent of the total gross domestic product (GDP) of Nepal (DFAMS, 1990). The most important livestock products are milk and meat, comprising 46.5 and 27.6 percent of the livestock gross domestic product (LGDP).

Additionally, livestock are an integral part of the crop production system, providing almost all the draft power used in cultivation, and most of the fertiliser to maintain the soil fertility. Livestock, mainly oxen and male buffaloes, are also widely used in hauling and transport of agricultural input and products. In the hills and mountains mules, yaks, goats and sheep make an important contribution as pack animals. In addition, since firewood is in short supply in many areas of the country, dung serves as fuel for cooking and heating as well as for fertiliser.

1.1.6 *Livestock productivity*

There are about 6.3 million cattle, 3.0 million buffaloes, 5.4 million goats, 0.9 million sheep, 0.3 million pigs and 11.6 million chickens in Nepal (DFAMS,1990). Cattle, buffaloes and goats are maintained by the most farm families. The rural household keeps on average 1.6 buffaloes, 3.8 cattle and 2.2 goats. These figures indicate that Nepal has the highest livestock population per household and possibly per unit of cultivated area, too. The demand for livestock products, however, is not being met by domestic production. DFAMS (1990) reported that food aid to Nepal in 1988 included 1700 tonnes of skimmed milk powder and 279 tonnes of butter oil. The export of livestock and livestock products (excluding woollen goods) in 1987-88 amounted to Rs.503 million, and the import of livestock and livestock products (excluding wool) amounted to Rs. 549 million in the same year.

The failure of such a large population of animals to meet the nation's requirement is largely due to their poor productivity. Typically the lactation milk yields of cattle and buffaloes are 836 and 455 kg respectively, the growth rates of goats to one year of age is 31 g per day, and the egg production of chickens is 80 eggs per year (Gatenby, Mahato and Shrestha, 1990). There are various factors responsible for limiting livestock productivity which need to be identified, analyzed and resolved. There is no doubt, however, that poor health is one of the important contributory factors which reduce the productivity of animals in Nepal.

1.1.7 *Animal diseases*

In general, disease diagnostic facilities in the country are insufficient for the needs resulting in there being a severe shortage of information about the prevailing diseases. However, rinderpest, haemorrhagic septicaemia, black quarter, swine fever, Newcastle disease and helminthoses have been identified as the diseases of major importance. It has been estimated that due to these major diseases the total loss to the national economy will be about 384.4 million rupees in the year 1995 (Anon, 1979).

Among the diseases of ruminants, fasciolosis is probably the most common disease, and perhaps, one of the important causes of livestock deterioration in Nepal. The disease is widespread throughout the country affecting all species of ruminant livestock; including yaks and yakows of the Himalayas (Joshi and Tewari, 1975). The different local names of this disease, such as *Namle, Mate, Lew* etc., in different regions, are proof of its continued existence for many years in the animal population of the country. In 1973, Singh, Basnyat, Eichenberger and Bommeli (1973) reported an infection rate of 50 to 90 percent in animals in areas below 1800 m and estimated an annual economic loss of Rs. 200 million (US$ 20 million) due to this disease alone.

1.2 Rationale and Objectives of the Study

1.2.1 *Epidemiological studies*

Despite the considerable economic impact and the endemic nature, there is a shortage of information about the epidemiological pattern of fasciolosis in the different climatic and agro-ecological conditions, and its relation with the various animal management systems practised in the country. The species of *Fasciola* and their snail vectors are not accurately identified. The ecology of the snail vector is not known. Furthermore, the importance of the disease in terms of mortality and indirect losses due to reduced weight gains and decreased productivity has not been assessed. The experience of other countries indicates that it is possible to reduce the problem to a minimum acceptable level if control programmes are based on a sound knowledge of these important aspects of the disease, and are implemented strategically, in an organised manner. Due to lack of this essential information, however, the Nepalese government's efforts over the last two decades to control fasciolosis by means of a drenching programme have been unsuccessful.

During the last two decades, several workers have attempted to determine the prevalence of fasciolosis in different parts of the country (Singh et al., 1973; Lowcock, 1982; Morel, 1985; Joshi, 1987; Joshi, 1988; Oli, Ratala and Basnet, 1989; Thakuri and Mahato, 1990; Mahato, Rai and Karki, 1991; Shrestha, Thakur, Dhakal and Mahato, 1992). Except for the study conducted by Morel and Mahato (1987), no comprehensive investigation has been made to establish the epidemiological cycle of this disease in Nepal. However, these authors carried out the study in the Koshi hills on cattle in one Village Development Committee (VDC) area only. It is not appropriate, therefore, to extrapolate their findings to other parts of the country because of the existance of wide variations in physiographic and agro-ecological conditions. Also, there is considerable variation in the management systems practised for different species of animals.

This study was undertaken, therefore, to establish the epidemiological dynamics of fasciolosis in eastern Nepal. Attempts have also been made to determine the epidemiological conditions, which give rise to the development of this disease in the area. In addition, it is hoped that the study will yield data that could be used to assess the economic significance of fasciolosis in the national economy.

1.2.2 *Pathogenesis studies*

Variation in breed susceptibility of animals to *F. gigantica* has been reported by several workers. Bitakaramire (1973a) showed in Kenya that the small zebu had a lower prevalence of the disease than exotic animals or the large zebu. Similarly, Castelino and Preston (1979) reported that Boran cattle had lower prevalence than Aberdeen Angus, Friesian x Boran or Herefords while the Aberdeen Angus had lower rates than the last two. There is also evidence that some breeds of sheep are more resistant than others. For example Ogunrinade (1984) found a higher level of resistance to *F. gigantica* in African dwarf sheep than Hammond (1973) reported in a Merino x Corriedale. Wiedosari and Copeman (1990) concluded from such measurements as the percentage take after experimental infection, the severity of anaemia and the values of serum enzymes that Javanese thin-tailed sheep have a high innate resistance to *F. gigantica.*

Boray (1966) considers that *F. gigantica* may have evolved from the more stable *F. hepatica* but that the speciation of *F. gigantica* was not complete. He also thinks that the diversity of the fluke may have been induced by adaptation to *L. auricularia* and to this snail's environment. It is possible, therefore, that the invasiveness and pathogenicity of *F. gigantica* may also vary within its very wide geographical range.

These reports suggest that pathogenesis studies using local breed of animals and local strain of the parasite are essential if true veterinary importance and economic impact of fasciolosis have to be established in an area. In Nepal, where fasciolosis is caused mainly by *F. gigantica*, no pathogenesis study has been carried out. Furthermore, information on pathogenesis of fasciolosis in buffaloes are scanty, though the prevalence of the disease is highest in this animal in Nepal as well as in other Asian countries. Therefore, controlled pathogenesis studies were undertaken to record some of the pathogenic effects of *F. gigantica* infection in indigenous Nepalese buffalo, goat and sheep.

1.2.3 *Molecular biology*

There have been limited studies on identification of the species of *Fasciola* existing in Nepal. Lohani and Jaeckle (1981/82) collected fluke specimens from 22 buffaloes and two goats at five slaughter places of Tansen, Palpa district located in the hills of western Nepal. As per the identification done by Hoerning Institute of Parasitology, University of Bern, they reported that *F. gigantica, F. hepatica* and an intermediate form occur in the survey area. Joshi (1989) found mixed infection of *F. hepatica* and *F. gigantica* in the buffaloes purchased from highland area in the western Nepal. In a survey in the Koshi hills of eastern Nepal, however, Morel and Mahato (1987) identified only *F. gigantica* on the basis of egg measurements and morphology of the adult flukes collected from the infected animals. Furthermore, they detected *F. gigantica* but not *F. hepatica* rediae on dissection of the lymneid snails collected in the survey area.

It is clear from the literature that the different species of *Fasciola* show variations in the intermediate host requirements (Kendall, 1965) and final host pathogenicity (Sewell, 1966). Therefore, correct identification of the species is desirable if epidemiological studies are to be meaningful in formulating control programmes.

Although several methods for differentiating *Fasciola* species have been developed, their identification is still based mainly on the morphological characteristics. The morphological differentiation of the genus *Fasciola* into distinct species is often difficult and so is unreliable. It is complicated not only by the fact that methods of fixation affect the shape, form, size (Hammond, 1970) but also many populations are found in which morphological characteristics do not conform to criteria established for recognised species (Kimura, Shimizu and Kawano, 1984). Other available methods also have their limitations, which are reviewed in detail in Chapter 10.

Within the past decade, the application of molecular biological techniques such as deoxyribonucleic acid (DNA) sequencing, repetitive DNA probes and restriction enzyme analysis of repetitive DNA fragments to the investigation of parasitic diseases has provided additional means by which diagnosis and phylogenetic studies can be performed. Molecular approach probably represents the definitive method of identifying many parasites and microorganisms since the DNA is the 'ultimate blue print' or plan of that organism (Harrison, 1991). Available information suggests that there are no profound DNA rearrangements between the various life cycle stages of parasites. So that the same DNA is present in a parasite whether it is in its definitive, intermediate or vector hosts or indeed in its free-living stages. The race and species specific DNA probes cloned from more easily available stages of parasite may be used to detect and/or define species, races and subspecies or rarer and less easily obtained stages of parasite (Harrison, 1991). Nucleic acid probes have now been developed successfully for the identification of many helminth parasites at species, sub-species and race levels (McManus and Simpson, 1985; Harrison, Delgado and Parkhouse, 1990; Garate, Harnett and Parkhouse, 1990). However, there is a dearth of nucleic acid probes for the identification of *Fasciola* spp. (Blair, 1993).

The purpose of this study was, therefore, to generate the DNA probes based on species-specific and common genomic DNA sequences of *F. gigantica* and *F. hepatica*. The species-specific probes could then be used to differentiate these two species by dot-blot or slot-blot analysis and the cross-reactive probes could be used for studying both inter and intra-specific variability in *Fasciola* spp. on the basis of their restriction length polymorphism.

PART ONE

EPIDEMIOLOGICAL STUDIES

CHAPTER TWO

LITERATURE REVIEW

FACTORS AFFECTING THE EPIDEMIOLOGY OF FASCIOLOSIS

2.1 Introduction

Fasciolosis, also refered to as liver fluke disease, is caused by trematodes belonging to the genus *Fasciola* Linnaeus, 1758. The earliest references to this disease were those of Jehan de Brie (1379) in his work Le Bon Berger (The Good Shepherd) (cited by Reinhard, 1957). Explosive outbreaks of fatal fasciolosis were documented in sheep in Europe as early as the 18th century (Ollerenshaw and Smith, 1969), but greater historical detail of the fluke and its life cycle was provided by Reinhard (1957).

Adult *Fasciola* normally inhabit the bile ducts and gall bladders of a wide range of mammals including man and wildlife species. The major domestic animal hosts are sheep, goats, cattle and buffalo.

The life cycle of the parasite involves an alteration of generations and requires a suitable species of *Lymnaea* snail, as an intermediate host. Eggs passed out from the infected animals hatch and the resulting miracidia infect the molluscan host. Following asexual multiplication as sporocysts and redia, cercariae are released and encyst as metacercariae on herbage. Encysted metacercariae are swallowed by the animal and the young flukes excyst in the gut and migrate through the gut wall and across the peritoneal cavity to penetrate the liver capsule. Then they migrate through the liver parenchyma for some weeks before entering the bile ducts where they mature.

Thus, before there can be any possibility of the life cycle of *Fasciola* species occurring in any particular area the following conditions must be satisfied. There must be an initial presence of infected final hosts, the intermediate snail host must be present and there must be an opportunity for transmission of the parasite from the final host to the snail habitat and for its return (Ollerenshaw, 1973). The success of the life cycle of *Fasciola* spp. depends on the development and hatching of eggs, infection of the snail host by a miracidium, development of larval stages within the vector, cercarial emission from the snail and formation of metacercariae on herbage, and finally establishment within the final host (Troncy, 1989). Completion of such phases of cycle eventually depends on how successfully the parasite can overcome complex interactions continually present in the environment and host. The factors which affect these stages in the life-cycle of *Fasciola* species, and thus influence the epidemiological dynamics such as the intensity of parasite population, and incidence and prevalence of the infection are subject of this review.

2.2. The Parasite

2.2.1 *Distribution of Fasciola species*

Among the several species of *Fasciola* (described in detail in Chapter-10), *F. hepatica* and *F. gigantica* are considered to be of major importance because the disease caused by these two species is an almost worldwide problem. Fasciolosis due to *F. hepatica* occurs mostly in the temperate climates of Europe, North America, North Asia, Australia and North Africa. The highland areas of Kenya, Ethiopia, South Africa, Central and South America, which are effectively temperate, also harbour this species together with many islands including New Zealand, Tasmania, Iceland, Cyprus, New Guinea, the Philippines and some islands in the Caribbean (Malek, 1980).

Fasciolosis caused by *F. gigantica* is more prevalent in tropical environments. It is widespread through Africa, Asia, and Hawaii (Losos, 1986).

There are areas where the distribution of *F. hepatica* and *F. gigantica* overlap. Pakistan (Kendall, 1954), India (Sharma, Dhar and Raina, 1989), Iran (Sahba, Arfaa, Farahmandian and Jalali, 1972), the Philippines (Kimura, Shimizu and Kawano, 1984), Japan (Akahane and Oshima, 1976), and Nepal (Singh et al., 1973; Lohani and Jaeckle, 1981/1982) are examples of areas where the two species co-exist. It is interesting to note that on the periphery of the distribution of *F. hepatica* and *F. gigantica*, an intermediate form between these two species has been reported from Japan (Akahane and Oshima, 1976) and Nepal (Lohani and Jaeckle, 1981/1982). In the area where zoo-geographical zones are in contact, hybridisation of *F. hepatica* and

F. gigantica is likely to occur because of existence of the mixed infections in animals for a long time (Hammond, 1970).

2.2.2 *Development and hatching of eggs*

The bionomics of *F. hepatica* eggs and miracidia have been studied by several workers (Rowcliffe and Ollerenshaw, 1960; Pantelouris, 1965) and detailed reviews have been produced by Boray (1969), Ollerenshaw and Smith (1969) and Soulsby (1982). Relevant data on *F. gigantica* were provided by Dinnik and Dinnik (1959; 1963) from studies carried out in the Kenya Highlands, at an altitude of about 2200 m. In general, flukes eggs are protected from desiccation and other adverse climatic factors by faeces. However, no significant development occurs as long as they are in contact with visible faecal material. Therefore, breaking up of host faeces and liberation of the eggs is essential for development and hatching to occur, provided also that a film of moisture is maintained over the surface of the freed eggs (Ollerenshaw and Smith, 1969; Ollerenshaw, 1974).

Rowcliffe and Ollerenshaw (1960) showed that eggs of *F. hepatica* did not develop at temperatures below 9.5° C, however, Boray (1969) observed that the hatching time was 90-100 days at an average temperature of 5° C with a range of 1° C to 11° C. There was an increased rate of development at temperatures ranging from 10° C to 26° C, with development time decreasing from 60 days at 12° C to 12 days at 26° C. Development was inhibited at temperatures above 37° C. Thus, the eggs of this trematode are unable to develop and hatch during drought, ideal conditions being provided in cool temperate climates. During the summer, development and hatching times may also increase in stagnant pools with plenty of organic matter (Boray, 1969).

Development and hatching of *F. gigantica* eggs occur at higher temperatures. Development time is approximately 17 days at a constant temperature of 26° C (Dinnik and Dinnik, 1959) which is longer than for *F. hepatica* eggs at this temperature (Rowcliffe and Ollerenshaw, 1960; Boray, 1969). Development and hatching of *F. gigantica* eggs must take place entirely under water. These processes are, therefore, largely controlled by the water temperature and are highly susceptible to desiccation and also to low temperatures. Development times from egg to miracidium during the warm and coldest seasons in the Kenya Highlands were 52 to 70 days and 90 days respectively, and hatching of eggs did not take place when the lowest minimum and highest maximum temperatures were 5.5°C and 19.5°C respectively (Dinnik and Dinnik, 1959). However, under optimum conditions, such as exist in permanent habitats in more typical tropical areas where temperature is not a limiting factor, development and hatching of eggs take place faster and all the year round (Troncy, 1989).

2.2.3 *Infection of snail host by miracidium*

The host-finding ability of trematode miracidia plays a vital role in the transmission of the parasite in that the continuation of the developmental cycle is directly related to the efficiency of the process. The host-finding process represents a complex interplay between the physiological and behavioral activities of the snail and the miracidium. The process exhibits a sequential pattern due to adaptive miracidial responses to the snail host and to environmental stimuli. In phase I, miracidia respond to environmental stimuli such as light and gravity in a manner similar to the host snail with the result that the miracidia concentrate in that part of the environment where a suitable host is likely to be found (Kendall and Parfitt,1953; Taylor, 1964; Chernin and Dunavan, 1962; Shiff, 1974). Phase II, the scanning phase, consists of random movements within the host environment, and phase III, the final localization and penetration of the host snail, is governed by the chemosensitivity of the miracidium to the host (Christensen, Nansen and Frandsen, 1976a). It has been suggested that the initial attachment and penetration of the epithelial cells of the snail host is performed by the miracidium while the final entry into the snail is accomplished by the young sporocyst (Dawes, 1960).

A limited pool of incorporated glycogen is the main source of energy during the free-living stage of the miracidium (Bryant and Williams, 1962). The declining speed of miracidial movement at increasing age, as demonstrated by Wilson and Denison (1970) for *F. hepatica* reflected a decline in available glycogen. They demonstrated an increasing rate of movement of the *F. hepatica* miracidium at increasing temperatures resulting in the commonly observed declining miracidial longevity at increasing temperature. However, such observations may be of limited value as Upatham (1972a) for miracidium of *Schistosoma mansoni* showed that motility/longevity is in general retained for a longer period than infectivity. Indirect evidence for a shortening of the infective period of the *F. hepatica* miracidium at high temperatures was obtained by Wilson and Taylor (1978), while direct evidence was obtained by Christensen, Nansen and Frandsen (1976b) who demonstrated that the penetrative capacity of the *F. hepatica* miracidium is unaltered for 7 hours at moderately high temperature and for 24 hours at 8°C.

Christensen et al. (1976b) observed that the lowest temperature level for the infection of the host snails by *F. hepatica* miracidia when in close contact with the snails was 5-6°C while the optimum temperature was 15-26°C. Although, the host finding process was slowed down at 10°C and 13°C, the level of efficiency remained identical to that at optimum levels, and the blockage below 5-6°C was reversed when the temperature was increased. Thus, the increase in the length of the scanning period of the *F. hepatica* miracidium at moderate low temperatures may to a certain extent compensate for the reduced host-penetration capacity at these temperatures.

Studies on the host-finding capacity of schistosome miracidia in relation to water volume, water depth and water velocity have shown that the ability to locate the host snail is in general reduced at increasing water volume, at increasing water depth and at increasing water velocity (Chernin and Dunavan, 1962; Shiff, 1968, 1969; Upatham, 1972b), but no such study has been conducted with *Fasciola* miracidia. Wilson and Taylor (1978) found that the host-finding capacity of *F. hepatica* miracidia was reduced at decreasing snail densities.

Christensen, Nansen and Frandsen (1978) conducted an experiment to investigate the influence of some physico-chemical factors on host-finding capacity of *F. hepatica* miracidia. They showed that the ability of the miracidium to penetrate the host snail (close confinement) is unaltered within the pH range of 5.4-8.4, but reduced at a pH of 8.9. They demonstrated that with close contact between miracidium and snail, infectivity is unaltered up to 2.79% NaCl followed by a progressive reduction at higher levels. Also, they found that the host-finding capacity of *F. hepatica* miracidium is reduced in water with high turbidity levels.

2.2.4 *Parthenogenetic development of larval stages in the snail*

Parthenogenetic development of the subsequent larval stages is closely linked with the biology of the snail vector. The latter provides an ideal environment for rapid multiplication of the parthenitae through polyembryony. The reproductive potential of digenetic trematodes is so enormous that a single miracidium is theoretically capable of giving rise to millions of cercariae (Troncy, 1989). Boray (1982) has estimated that approximately 4 to 5 generations of *Fasciola* cercariae can develop in a year in a tropical climate and about 4 in tropical highlands and subtropical climates. This contrasts with about 1 to 2 generations in temperate climates (Soulsby, 1982).

In Australia, Boray (1963) studied the effect of temperature on the development of *F. hepatica* in *L. tomentosa*. Below 10°C the larval stages of *F. hepatica* survived for at least 100 days without completing their development, and without causing apparent damage to the snails. Larval development was completed and large number of metacercariae were produced when temperature was raised to 20°C. One snail population survived for 3 years at 2-5°C. He suggested that these larvae may survive much longer at low temperatures than is necessary for overwintering, which may explains the possibility of survival by the parasites in northern Europe, Scandinavia, the Baltic area and some parts of Siberia, where one generation of metacercariae may take 2-3 years to develop. Boray (1963) showed that the increase of temperature decreased the development time of *F. hepatica* parthenitae. However, he further observed that higher temperatures during development may affect the viability and infectivity of metacercariae; the infectivity of metacercariae decreased slightly at 26°C and substantially at above that temperature, but some metacercariae produced at 35°C were infective to sheep.

Accumulation of larval stages in aestivating and hibernating snails also provides an effective means of parasite survival during adverse climatic conditions and a guarantee of successful transmission when environmental conditions improve, particularly in the case of *F. hepatica* in cool temperate and subtropical climates (Boray, 1969).

Dinnik and Dinnik (1963) have estimated that development of the larval stages of *F. gigantica* in *L. natalensis* under field conditions in Kenya would be completed in 69 to 197 days, depending on the seasonal temperature. In aquaria maintained at a constant and more suitable temperature of 26°C it took a minimum of 33 days. Since the development from egg to miracidium under similar conditions took 17 days (Dinnik and Dinnik, 1959), the whole process from egg to cercariae at this temperature was approximately 50 days. Dumag, Batolos, Escandor, Castillo and Gajurado (1976) observed that intramolluscan development of *F. gigantica* in *L. rubiginosa* required a minimum of 44 days and a maximum of 68 days under laboratory conditions in the Philippines.

2.2.5 *Cercarial emission from the snail and formation of metacercariae*

Standen (1963) reported that the *L. natalensis-F. gigantica* association does not respond as well as the *L. truncatula-F. hepatica* one to the reduced temperature stimulation technique for cercarial emergence. He found that the cercariae were frequently discharged at night. Hammond (1970) observed that *F. gigantica* cercarial emergence was pronounced soon after the snails were removed from daylight into the dark incubator. On the other hand Taylor (1964) stated that the shedding of cercariae of *F. gigantica* was induced by direct sunlight, or the light from a 60 watt electric bulb. On the basis of these findings Hammond (1970) suggested that darkness is but one of the stimulants to the emergence of cercariae, rather than that there is a form of periodicity or biological rhythm involved. He further suggested the possibility of different stimulants requirement by different physiological races of flukes for the emergence of cercariae.

Kendall and McCullough (1951) observed that 9°C was the critical temperature below which emergence of *F. hepatica* from *L. truncatula* did not occur. The cercariae emerged equally at 26°C or at a temperature (10°C) which was only slightly above the critical minimum. Their further observations showed that emergence might occur throughout a wide range of intermediate temperatures and under conditions of rising or of falling temperatures. The effect of temperatures higher than 26°C was not observed , however, they had no evidence that emergence was inhibited at high temperatures. Marked fluctuations in climatic factors in the snail habitat induce spontaneous emission of cercariae, especially by aestivating snails and the resultant sudden increase in herbage metacercarial infestation may, in some situations, give rise to major disease outbreaks (Boray, 1969).

Cercariae encyst within a few hours of their leaving the intermediate host and the process of encystment requires warm and wet conditions. Although encystment may occur on any object, cercariae of *F. gigantica*, in particular, encyst on fully submerged vegetation or herbage. Dumag et al. (1976) reported that the cercariae of *F. gigantica* encysted on parts of plants at least about two centimetres below the water and that no metacercariae were found on parts of the grasses above the water level. They further observed that no cercariae were found encysted on parts of grasses very close to the soil level or on the surface of water or slightly below the water level. The green parts of the grasses were preferred by the cercariae as the site of encystment, indicating that the cercariae of *F. gigantica* were attracted by the green colour of plant.

Occasionally encystment may occur on the water surface (Dinnik and Dinnik, 1959). In this situation some of the metacercariae may sink to the bottom of the body of water, becoming available when stirred up by the final host as it wades into the water to drink (Soulsby, 1982). However, Boray (1963) found that about 10% of cercariae of *F. hepatica* encyst on the water surface by enclosing small airbubbles which enable them to float for long periods and thus infect hosts through drinking. Similarly, Esclaire, Audousset, Rondelaud and Dreyfuss (1989) reported that free-floating metacercariae which represented 6.8% of metacercariae produced by *L. truncatula* infected with *F. hepatica*, were able to float on the water surface for more than three months. The authors suggested that such cysts may be responsible for human infections reported after drinking river water.

2.2.6 *Survival of metacercariae*

The survival of metacercariae has important implications from the epidemiological point of view. Metacercariae have been shown to survive for more than one year under laboratory conditions but, under natural conditions, it is likely that a dangerous level of infection does not persists for such a long period (Soulsby, 1982).

Boray and Enigk (1964) carried out laboratory experiments using both *F. hepatica* and *F. gigantica* at temperatures between -20°C and +35°C and at controlled relative humidities. They observed that metacercariae of *F. hepatica* were no longer infective, although apparently viable after 12 hours at -20°C . At -10°C most metacercariae retained their viability for 7 days and some were still infective at 28 days. Freezing in water at -10°C destroyed them, however, in 7-28 days. At -5°C some metacercariae were infective after 28 days but were non-infective after 56 days. If they were kept at -5°C for 12 hours in each day and at +10°C for the other 12 hours a high proportion was infective after 70 days. Metacercariae of *F. gigantica* were able to survive appreciably longer periods at high temperatures than those of *F. hepatica*. *F. gigantica* metacercariae survived 114 days and 21 days at 30°C and 35°C respectively, whereas those of *F. hepatica* died between 14 and 36 days and before 14 days respectively. The results obtained at different relative humidities confirmed the view that a high relative humidity (90% or more) is necessary for prolonged survival, particularly at higher temperatures. *F. gigantica* metacercariae were more susceptible to desiccation than *F. hepatica*.

The longevity of metacercariae under field conditions is limited as they are susceptible to desiccation and direct sunlight (Olsen, 1947). Alicata (1938) found that metacercariae attached to whole plants and kept in sunny areas were viable after 15 days in Hawaii but not after 42 days. Taylor (1949, cited by Boray, 1969) indicated that a large proportion of metacercariae fell off certain types of herbage within four to six weeks and though such forms may remain viable for some time they are generally unavailable to grazing animals. Metacercariae of *F. gigantica* can survive on stored fodder such as rice straw for up to 4 months (Kimura and Shimizu, 1978). However, this survival appears to depend on temperature, relative humidity and the moisture content of the stored product as observed by Abu and Shiramizu (1985) and Shiramizu and Abu (1988). In Nepal, Joshi (1986, 1989) found that *F. gigantica* metacercariae in the rice straw bundles which were kept in an open place, remained infective at least for two months. This suggests that in some situations stored fodder may constitute a potential source of *F. gigantica* infection.

Metacercariae accidentally ingested by snails are passed out in faeces, unaffected by the latter's digestive processes, and are fully infective for the definitive host (Boray, 1969; Yadav and Gupta, 1988). Although this is one possible method of re-cycling and passive dispersal of metacercariae within and between habitats, its epidemiological significance remains to be determined. A commoner and possibly more important method of passive dispersal of metacercariae is when cercariae which encyst on the water surface are transported for long distances by the current.

2.3 Host-parasite Relationships

Any herbivorous animal is a potential host for *Fasciola* spp, however, Boray (1969) has classified some common domestic and synanthropic animal hosts of *Fasciola* spp. into three broad groups on the basis of their susceptibility and response to infection. The first group, the early resistance group, is naturally resistant to the parasite, as their tissues are unsuitable for establishment and survival of the parasite which, as a result, is quickly eliminated with minimal or no pathogenic effects on the host. Animals in this group include the domestic pig, dog and cat. The infection in these animals is, therefore self-limiting.

The second group, the delayed resistance group, is moderately susceptible to infection which, however, is controlled in the early stages by a "mechanical resistance" and in the later stage of the infection by a delayed host fibrous tissue reaction. The profound alteration in liver architecture associated with this reaction ultimately leads to the elimination of any parasites reaching the bile ducts, by imposing a "mechanical barrier" to their feeding. The host, nevertheless, may show severe liver pathology resulting in high morbidity and mortality, particularly in young and malnourished animals. The parasite readily completes its life-cycle and can be successfully transmitted to other susceptible hosts. The infection in this group is also self-limiting. Members of this group include cattle, water buffaloes, horses, donkeys, mules, asses and man.

The third group, the low resistance group, is highly susceptible to infection and shows severe, early (acute) and delayed (chronic) tissue reaction but with insufficient hepatic and biliary fibrosis and cellular response to interfere with the development and survival of the parasite. Usually, all ages of animal are susceptible and light infections may survive as long as the hosts. Both the acute and chronic stages of the disease are often accompanied by marked clinical signs and significant mortality. Members of this group include sheep, goats, rabbits, rats and mice.

The above three broad categories give an indication of the potential roles of each group of hosts in the life-cycle, maintenance and transmission of infection. Thus, while most of the hosts in group one are largely irrelevant in the transmission of infection, those in group two and three are crucial to the successful life-cycle and transmission of the parasite. The high prevalence rate and the pathogenic effects in such hosts as sheep, goat, cattle (Hammond, 1965; Sewell, 1966; Hammond 1970) and buffalo (Swarup and Pachauri, 1987a; Swarup, Pachauri and Mukherjee, 1987) account for the world-wide economic importance of the parasite. Other hosts in these groups such as the rabbit, horse, donkey and camel may, in specific situations, act as important reservoir host. The role of the rabbit as a reservoir host with very long-lived infection is well documented (Taylor, 1964).

A large number of wildlife, such as African buffalo (*Sncerus caffer*), Uganda kob (*Kobus kob*), hartebeest (*Alcelaphus buselaphus jacksoni*), and giraffe (*Giraffa camelopardalis*) have been shown to be susceptible to infection with both *F. hepatica* and *F. gigantica* (Bindernagel, 1972; Hammond, 1972). *F. gigantica* has also been found in the liver of Indian rhinoceros (*Rhinoceros unicornis*) (Bhattacharjee and Halder, 1971) and in warthog (*Phacochoerus aethiopicus*) (Troncy, Graber and Thal, 1973). It would seem that these wildlife could be efficient reservoir hosts (Hammond, 1972).

The egg production capacity and life-span of *Fasciola* in the definitive host are a reflection of the susceptibility and resistance of the host. *F. hepatica* normally live for about 9 months in sheep before they die or are expelled from the host but longevities of 8 to 11 years have been reported (Boray, 1969; Soulsby, 1982). The mechanism responsible for this prolonged survival is unknown. None of the various "avoidance mechanisms" which could possibly enable the parasite to survive in its host such as acquisition of host antigens, turnover of parasite glycocalyx to prevent antibody attachment and/or cell adherence and production by parasite of substance or substances which can prevent host effector cells from functioning, appear to be implicated (Hughes, 1987).

Boray (1969) estimated the daily faecal egg output in light subclinical *F. hepatica* infection in sheep to be 0.5 million. Animals with moderate infections shed 2.5 to 3.0 million eggs daily in their faeces. Hammond (1973) also noted that large number of eggs were passed in the faeces of the sheep subclinically infected with *F. gigantica*. Thus, the level of pasture contamination is considerable, even in subclinical infections. This high fecundity of the parasite, the high reproductive capacity of the snail vector and polyembryony of larval flukes in snails are responsible for the rapid recolonisation of habitats and contribute to the successful maintenance and spread of infections in endemic areas. Furthermore, in those situations in which highly susceptible hosts are exposed to repeated waves of infection, lack of synchrony in attainment of maturity of flukes derived from the succession of infective stages, and the prolonged parasite survival in the host, would tend to prolong very considerably the duration of patency and increase the intensity of pasture contamination. The same lack of synchrony in the maturation of flukes also occurs in single infections (Soulsby, 1982).

Egg production by *Fasciola* spp. in cattle is less persistent than in sheep and goats due to the limited patent period of infections (7 to 9 weeks in moderate and 13 to 15 weeks in heavy *F. hepatica* infections), lower fecundity and more rapid elimination of flukes (Dickson, 1964; Boray, 1969). Under Australian conditions the daily faecal egg production of cattle chronically infected with *F. hepatica* and having a faecal egg count of only 5 eggs per gram (EPG) was approximately 20 times less than that of similarly infected sheep (Boray, 1969). Nevertheless, in cattle, buffalo and other large animal hosts such low EPG is epidemiologically important and can contribute significantly to pasture contamination during the patent period, in view of the relatively large volume of faeces produced by these hosts.

These relationships between *Fasciola* and its definitive host are strongly influenced by several factors, the most important of which are the resistance status, age and nutritional plane of the host (Boray, 1969; Schillhorn van Veen, 1974; Hughes, 1987) and concurrent infections (Reid, Armour, Jennings, Kirkpatrick and Urquhart, 1967; Schillhorn van Veen, 1974). In rats and mice the sex and genotype of the host also appear to be important (Boray, 1969; Hughes, 1987).

Laboratory animals, notably rats and mice are able to control primary infection with *F. hepatica* and subsequently develop strong resistance to reinfection (Hughes, 1987). This resistance can be transferred to recipient naive animals by immune serum or cells (Lang, 1967; Lang, Larsh, Weatherby and Goulson, 1967; Armour and Dargie, 1974; Corba, Armour, Roberts and Urquhart, 1971). Rats which have been infected orally with metacercariae for a short time (2 to 3 weeks) are found to be resistant to challenge either by the oral or peritoneal route (Hughes, Harness and Doy, 1977). The resistance to infection wanes with time and after about 6 months the rats are no longer resistant, but this ability is quickly restored within two weeks if the rats are reinfected. Resistant rats can kill metacercariae given orally, newly excysted juveniles and also adults placed in the peritoneal cavity. The killing occurs at the gut wall within 48 hours of challenge (Hayes and Mitrovic, 1977; Hayes, 1978) and there is evidence that antigens common to the three stages of fluke are involved in the protective immunity.

Although the evidence for acquired resistance to infection in small laboratory animals is incontrovertible, this is far from the case in domestic animals. So far, it has not been possible to establish a role for immunologically mediated resistance in the control of *Fasciola* infections, particularly in sheep and goats where attempts to induce immunity through the administration of irradiated metacercariae, *Fasciola* antigens and other procedures have been unsuccessful (Hughes, 1987). The current situation was summarised by Hughes (1987) who stated that "no worker or group of workers have to date been able to unequivocally prevent reinfection in sheep by immunological means".

Cell-mediated immune response, assessed by means of *in vitro* lymphocyte proliferation assay and interleukin (IL_2) production, has been demonstrated in bovine fasciolosis (Oldham and Williams, 1985). Also, encouraging results have been reported by several workers who have attempted to immunise this host by using irradiated metacercariae (Bitakaramire, 1973b; Armour, Dargie, Doyle, Murray, Robinson and Rushton, 1974; Nansen, 1975; Younis, Yagi, Haroun, Gameel and Taylor, 1986) or by transfer of antiserum or primed lymphoid cells (Corba at al., 1971; Dargie, Armour and Urquhart, 1973). There is, however, still insufficient evidence to show that resistance to infection, which undoubtedly occurs in both experimental and field infections in this host is immunologically mediated. It is generally believed that the resistance is associated with the marked alterations in liver and bile duct architecture resulting from liver damage caused by migrating flukes and damage of the bile ducts by adult flukes (Hughes, 1987). However, Dargie et al. (1973) have shown that in monozygotic twin calves resistance to re-infection can develop in the absence of liver damage. Nevertheless, one must conclude from the available evidence that immunologically mediated (acquired) resistance is unlikely to play an important role in the epidemiology of naturally occurring bovine fasciolosis, but that non-immunological resistance may have a limited role in this and other naturally relatively resistant hosts through its influence on parasite fecúndity, growth and survival.

In cattle, heavy infections and clinical disease occur most commonly in calves and resistance to challenge infection, which usually develops following either single primary or continuous low level infections (Boray, 1969) is stronger in adult than in young animals. This contrasts with the situation in sheep and goats, adults of which are fully susceptible to infection and readily develop clinical disease (Schillhorn van Veen, 1979; Ogunrinade, 1984).

The nutritional plane of the host has a broadly similar effect on body resistance in most susceptible hosts. Experiments in which groups of lambs fed different rations were infected with a standard dose of metacercariae showed that those on a low plane of nutrition died much sooner after heavy infections and developed more profound anaemia than those on a high plane of nutrition (Boray, 1969; Berry and Dargie, 1976; Dargie, Berry and Parkins, 1979). This was attributed to increased "state of resistance" of the well-nourished animals. Schillhorn van Veen (1974), working in Northern Nigeria, reached a similar conclusion based on field observations on nomadic and semi-nomadic cattle during severe drought. His observations highlighted the interactive influences of malnutrition, long distance trekking by migrant herds and concomitant parasitic and non-parasitic infections on the incidence of fasciolosis.

Little information is available on the role of concurrent infections on the epidemiology of fasciolosis in the tropics. The best known examples of naturally occurring associations between *Fasciola* and other pathogens in livestock are the interaction between *Fasciola* and *Clostridium novyi* especially type B in the causation of necrotic hepatitis or black disease (Bagadi, 1970; Bagadi and Sewell, 1973; Bagadi, 1974; Soulsby, 1982; Samad and Haqub, 1987), that between *F. hepatica-Ostertagia ostertagi* complex in some temperate climates (Reid, Armour, Jennings, Kirkpatrick and Urquhart, 1967). Fasciolosis has been suggested as precipitating factor in the causation of bacillary haemoglobinuria, a fatal disease of animals caused by *Cl. novyi* type D, but evidence on this point is incomplete (Blood and Radostits, 1989). In these associations, fasciolosis usually enhanced the pathogenicity and/or the persistence of the other pathogens in the host, except that in the fasciolosis-ostertagiosis complex mutual enhancement of pathogenicity probably occurs.

Concurrent infections of cattle with *Fasciola* and *Schistosoma* appear to elicit heterologous resistance. Yagi, Younis, Haroun, Gameel, Bushara and Taylor (1986) have shown that mature *F. gigantica* infections in zebu calves can induce significant resistance to challenge infection with *S. bovis* cercariae and vice versa. Some non-immunologically mediated resistance to *Fasciola* has also been observed in sheep concomitantly infected with *Cysticercus tenuicollis* (Campbell, Kelly, Townsend and Dineen, 1977). However, the latter observation was not confirmed by Hughes, Harness and Doy (1978). In view of the well known immunomodulatory properties of helminth and protozoan parasites and the fact that multiple parasitic infections are the more natural forms of parasitism in tropical livestock, it would be surprising if concurrent infections did not have some modulatory influence on the course of fasciolosis in some natural host-parasite systems.

2.4 The Intermediate Hosts

2.4.1 *Distribution of the species of snail vectors*

After the life history of *F. hepatica* was described by Leuckart and Thomas independently in 1882 (cited by Taylor, 1964) and it was shown that this involves a snail, a number of species of molluscan intermediate hosts of *Fasciola* spp. have been reported. However, Kendall (1965) believed that the vectors of *Fasciola* spp. are limited to species of *Lymnaea* and transmission depends on a limited number of species and usually in each area only one species is involved.

Kendall (1965) suggested that, throughout the greater part of the world, *F. hepatica* is transmitted by *L. truncatula*, an amphibious snail living on wet mud at the side of shallow water and never in deep permanent water, or by snails which are not readily distinguishable from it, either on morphological grounds or on the grounds of their ecological requirements. In respect of *F.gigantica*, he suggested that snail hosts are true aquatic belonging to Hubendick's (1951) superspecies *L. auricularia*. The different species of snails reported to be the intermediate host of *Fasciola* are tabulated by Soulsby (1965); only those which are known to be important are considered here.

L. truncatula is the most important and widespread intermediate host of *F. hepatica* in Europe, Asia and Africa. In North America, the principal intermediate host of *F. hepatica* is *L. bulimoides*, and in Australia it is *L. tomentosa*. Other species which have been been found to transmit *F. hepatica* include *L. viator* and *L. diaphena* in South America, *L. columella* in Central and North America, Australia and New Zealand, and *L. humilis* in North America. *L. auricularia sensu stricto* may be involved in the life cycle of *F. hepatica* in the Kashmir valley in India (Sharma et al., 1989).

The principal snail vector of *F. gigantica* in the Indian sub-continent is *L. auricularia* race *rufescens* (*L. acuminata* synonym) (Kendall, 1965). Other reported vector is *L. auricularia sensu stricto* (Prasad, Gupta and Chandra, 1987). In South East Asia, the vector has been identified as *L. auricularia* race *rubiginosa* (Kendall,1965). In Africa, the recognised vector of *F. gigantica* in both the south and the west is *L. natalensis* (Schillhorn van Veen, 1980), while in East Africa it is transmitted by *L. caillaudi* (Van Someren, 1946). *L. caillaudi* is now regarded as a synonym for *L. natalensis*, which in turn is not specifically distinguishable from *L. auricularia rufescens*, with which it merges in the region of Oman and Lower Mesopotamia (Hubendick, 1951)

In the Philippines, though, *L. rubiginosa* is the principal vector of *F. gigantica*, *L. viridis* has been found to act as the intermediate host at relatively high altitudes (Dumag, Batalos, Escandor, Castillo and Gajudo, 1976). In Burma, *L. viridis* is considered as a counterpart of *L. truncatula*, i.e. intermediate host of *F. hepatica* (Taylor, 1964). This species has also been found as an important intermediate host of *Fasciola* spp. in Nepal (Singh et al, 1973).

2.4.2 *The habitats and tolerance ranges*

The snail intermediate hosts of *F. gigantica* are truly aquatic and require permanent, still or slowly-flowing, clear bodies of water with abundant vegetation but little decaying organic matter (Hammond, 1965). Therefore, amphibious conditions in water courses or other aquatic habitats which disappear during dry spells are unsuitable. Under natural conditions, the ideal habitats are found in borders of permanent lakes, in streams, irrigation channels, flood-prone areas and low lying marshes (Troncy, 1989). By contrast, *L. truncatula*, the snail intermediate host of *F. hepatica* is basically an aquatic snail which has adapted itself to conditions which are not permanently aquatic (Ollerenshaw, 1973). Typical habitats include badly drained land and shallow marshy areas, temporary springs and water courses, irrigation ditches, areas of seepage of springs and broken drains. Even hoof prints of animals in clay soils as well as muddy areas in gateways and around drinking troughs may provide temporary habitats for this species of snail (Soulsby, 1982).

The distribution, frequency, size and suitability of habitats depend on the interaction of a number of physical, chemical and biological features in the environment (Ollerenshaw, 1974). Physical features such as topography, soil type and geological formation of an area influence the water drainage and retention. For instance clay soils are more moisture retentive than sandy soils, impervious rocks such as granite and slate tend to retain water in the overlaying soil layer as compared with porous rock. The topography of the land may facilitate or hinder natural drainage whilst man-made drainage may range from highly efficient to the non-existent.

It is difficult to interpret the biological significance of hydrogen-ion concentration (pH), since pH itself is bound up with many other factors such as the alkali reserve, salinity, free CO_2 and the amount and state of organic matter present in the water, any one or all of which may in themselves be determining factors (Van Someren, 1946; Macan, 1974). Boycott (1936) considered pH not to be a major factor in snail distribution and suggested that the word `Indicator', not `Controller'- sums up its function best.

The results of a study by Van Someren (1946) indicated that while *L. natalensis* in the Lake province and Highlands of Kenya is apparently tolerant of a range of pH 6.0-9.5, it is more frequently present in the range pH 6.5-8.0. A similar study in a natural habitat at Nishat in Kashmir, India showed that *L. auricularia sensu stricto* survived, bred and multiplied in an alkaline medium of a range of pH 7.12-9.04 (Dhar, Bansal and Sharma, 1985). Boray (1969) reported that *L. tomentosa*, the major snail vector of *F. hepatica* in Australia, thrives at a pH range of 6.0 to 7.0.

The presence of calcium in the water is probably of more importance than pH, since it is necessary for the construction of the shells. Dissolved calcium appears to be favourable to nearly all freshwater snails in Europe, and half of the species in Britain are hardly ever found in soft water, with less than 20 mg/l Ca (Boycott, 1936). Calcium may regulate the quality and quantity of aquatic vegetation and hence affect the snails indirectly (Van Someren, 1946). Poor food supply has been found to be associated with scarcity of molluscan species in soft waters (Macan, 1974). Schutte and Frank (1964) found that *L. natalensis* occurred in all the 4 categories of habitats, (1) very soft (0-10 ppm Ca), (2) soft (11-30 ppm Ca), (3) hard (over 31 ppm Ca) and (4) very hard (with alkalinity exceeding 150 ppm $CaCO_3$), though with generally decreasing frequencies from very hard to very soft. Observations in Kenya (Van Someren, 1946) also suggest that *L. natalensis* is tolerant of soft waters, as snails were found in nearly half of 49 waterbodies containing from 1 to 19 ppm $CaCO_3$.

A study of the distribution of *L. natalensis* near Nairobi and in western Kenya led Van Someren (1946) to believe that oxygen tension is probably a principle limiting factor for this species, and that in a favourable habitat O_2 tension should not fall below about 75%. In experiments, snails showed distress when saturation fell below 40%, and a saturation below 9% generally resulted in their death. Hunter (1953) made the relevant observation in England that *L. peregra* is obliged to come to the surface for air when the temperature exceeds 12°C, and for this reason is confined to the shallow water of some lakes. So far as known, *L. natalensis* is confined to the upper littoral zone in tropical lakes, and the explanation could be that high temperatures prevent this species from becoming independent of atmospheric air (Brown, 1980).

In recent years many observations have been published on salinity in inland waters, partly because this property can be rapidly measured as electrical conductivity. In fresh waters the common inorganic salts are sufficiently diluted to be almost entirely dissociated into their component ions, and the conductivity of the water reflects closely the total concentration of major ions. Like pH, conductivity is a measurement that expresses the cumulative effects of a complex of chemical and physical properties, and it should be taken as no more than a guide to the factors which actually determines the presence or absence of a species. An investigation of snail distribution on a large scale in the area of the Mangoky Irrigation Project, southwest Madagascar (Degremont, 1973) revealed that the sites in which no snails were found, or in which some were occasionally present, had comparatively high electro-conductivities with an average of 1005 μmhos. Boray (1969) reported that *L. tomentosa* in Australia, thrives at a salinity range of 25 to 160 ppm. A drastic reduction in snail population occurs with increasing salinity following evaporation.

Detailed observations on the biology of the snail vectors of *F. hepatica* in Australia (Boray, 1963; 1969) and Britain (Ollerenshaw, 1959; Ollerenshaw and Rowlands, 1959; Ollerenshaw, 1974) have shown that in temperate climates maintenance of wet conditions with an adequate balance between rainfall and evapotranspiration during dry periods, and plentiful food supply enhance the reproductive capacity, growth and survival of snails. The better the state of nutrition of snails, the larger their size and that of the larval fluke burden which they can support; large snails harbouring almost ten times the number of developmental stages found in smaller, malnourished ones (Kendall, 1949). Hence, the size, and not the number of snails, is the important factor in their vectoral capacity (Kendall and Ollerenshaw, 1963).

Dispersal of snails is an important aspect of their population biology and distribution within and between habitats. Lowered oxygen tension is apparently one of the factors which can initiate active migration of some species of amphibious lymnaeids out of stagnant pools, while their speed of migration depends on water temperature and stream gradient of the habitats (Boray, 1969). Studies on the Australian lymnaeid, *L. tomentosa*, showed that at temperatures of 4.5 to 6.2°C it migrated up to 150 m in approximately 12 weeks. At higher temperatures it travelled up to 150 m in 8 weeks. Snails can also migrate passively by water current (Rahman and Ed Din, 1961). In large-scale flooding, snails can be carried to new habitats hundreds of miles away from their origin (Boray, 1969). Birds and animals also play a big role in passive migration of snails, as snails or snail eggs can be carried on feet or plumage of birds (Boag, 1986), and may lodge on the hooves or in mud on the animal's body. Some freshwater snails may also pass through alimentary canals of birds (Malone, 1965). Furthermore, transport of freshwater plants either for commercial purposes or interchange of plants between botanical gardens or by private persons has often resulted in snail species passing natural barriers (Walker, 1978; Madsen and Frandsen, 1989). These means of transport allow snails to colonize new habitats and foci of infection within a short time (Madsen and Frandsen, 1989).

2.4.3 *Aestivation ability*

The ability of *L. truncatula* and other amphibious snails to survive in a state of dormancy during the winter (hibernation) and prolonged drought (aestivation) is another important factor in their biology and efficiency in transmission of infection (Soulsby, 1982). In temperate climates, hibernating and aestivating snails may survive in frozen or dried mud, as the case may be, for more than a year (Kendall, 1953). On the return of favourable environmental conditions such snails quickly repopulate their habitat due to their high biotic potential. This is also accompanied by increased production and release of cercariae as a result of enhanced development of the larval stages which had accumulated in the snails, thereby creating large herbage population of metacercariae (Boray, 1969; Soulsby, 1982). Equally important are short spells of drought followed by wet conditions and intermittent, sudden, marked fluctuations in climatic factors like temperature and moisture in the habitat of dormant snails. These influences can lead to massive emissions of cercariae and heavy fluke burdens in animals (Boray, 1969).

Aestivation may be central to the ecology of many snail species which act as intermediate host of *F. gigantica*, but the phenomenon has not been investigated extensively. Boray (1969) suggested that all lymnaeids may aestivate for a considerable time during dry periods, in which case the phenomenon may also be of epidemiological importance in the tropics. However, to the contrary, Soulsby (1982) stated that the snail vectors of *F. gigantica* can survive an amphibious existence but can aestivate for only very short periods.

In the laboratory, Cridland (1967) investigated tolerance of desiccation by *Bulinus globosus*, *B. africans*, *Biomphalaria pfeifferi* and *L. natalensis* in boxes containing different soils. *L. natalensis* was least successful at surviving on the surface, though some large individuals did so for up to 21 days, during a period when the temperature of the soil reached 35°C and its moisture content dropped to 8.8%. A larger proportion of the other species survived for up to 30 days at the surface. Also, *L. natalensis* gave the lowest of the survival rates when buried in earth. Similarly, in Tanzania, Hammond (1965) could not find any evidence that *L. natalensis* can aestivate which agreed with the observation of Coyle (1958) in Uganda. These results supported the view that this species is adapted to be primarily an inhabitant of permanent waterbodies, but, other observations made in natural habitats near Nairobi and in the laboratory showed that a proportion of *L. natalensis* can survive in hard dry mud for at least 24 weeks (Bitakaramire, 1968) indicating that capacity for aestivation may be well developed in particular populations of this snail.

In a study by Sharma and Bhat (1989), *L. auricularia sensu stricto* experimentally infected with *F. gigantica* were allowed to undergo hibernation in 10 cm deep layer of wet sand soil. After 120 days, 60% of the infected snails were found alive and remained positive for *F. gigantica* infection, indicating that over-wintering infection may play an important role in the epidemiology of fasciolosis caused by *F. gigantica*.

2.4.4 *Predators, pathogens, competitors and toxic plants*

There is a good evidence that several biological agents, including some species of algae, fungi, bacteria, nematodes and plants can adversely affect the survival, reproduction and/or growth of snails and attempts have been made to apply this concept in the biological control of snails vectors of trematodes (McCullough, 1981; Madsen, 1990). Although, some of these agents are efficient killers of snails and theoretically could influence the population dynamics of the vector, there is little evidence that they are of practical value under field conditions. Currently, biological control is largely experimental and geared towards the schistosome intermediate hosts.

Many invertebrate predators have been suggested as biological control agents, but only few groups, such as sciomyzid flies and certain leeches are specific snail predators; other predators tend to choose alternative food sources when snail density decreases, thus reducing the predation pressure on the snail population (Madsen, 1990). Vertebrate predators, in particular domestic ducks and geese may affect the density of freshwater snails in certain habitats as Coyle (1959) quoted one case where these birds were thought to be responsible for disappearance of various species of snails, including *Lymnaea* from 10 experimental fishponds.

Several fish species, such as *Serranochromis* spp., *Astatoreochromis alluaudi*, *Tilapia melanopleura*, *Clarias* spp. etc., have been reported to be malacophagous, but rigorous observations on their efficiency in field situations are still lacking (McCullough,1981). Gupta, Prasad and Chandra (1986) reported that *Channa punctatus*, a freshwater fish which may be cultured in derelict, weed-infected waters, was found to feed on *L. auricularia*. Laboratory studies showed the rates of predation to vary with the size of the fish and of the mollusc.

If two species of snails are sufficiently similar in their biological profile, then one inevitably eliminates the other weaker. This competitive exclusion/displacement approach to control snail vectors of *Schistosoma* spp. has received most attention recently. Among the several competitive species of snails, most interest has been focused on *Marisa cornuarietis, Helisoma duryi, Melanoides tuberculata, Pomaecea haustru, Potamopyrgus jenkinsi, Bulinus tropicus, Thiara granifera and Physa* spp. (McCullough, 1981, Madsen, 1990). So far, *M. tuberculata* and *T. granifera* have been found to be the most efficient competitors of *Biomphalaria glabrata* (Madsen, 1990; Perez, Vargas and Malek, 1991). Except for *M. cornuarietis*, however, there appears to be no such study been reported on competitor of *Lymnaea* spp.

In recent years, many molluscicides of plant origin have been reported in the literature (Kloos and McCullough, 1982; Agrawal, Bhandari and Chaudhry, 1987). Results of an investigation by Bali, Singh and Pati (1985) indicated that if some plant species, such as *Sapindus emarginatus, Acacia concinna, Caesalpinia corearia and Embelica officinalis* are planted on the banks of ponds, streams and near ditches on the sides of roads and canals, the fruits and leaves which fall into water bring about the natural control of different species of vector snails. Similar effects of Eucalyptus trees were noted by Coyle (1961) and Hammond (1970) suggesting that presence of plant species with marked molluscicidal properties around the edges of habitats may substantially affect the distributions and densities of vector snails.

2.5 Relationship Between *Fasciola* spp. and Snails

The susceptibility of lymnaeids to *Fasciola* spp. and the adaptability of the latter to their intermediate host are important considerations in the epidemiology of fasciolosis. Although the intermediate snail hosts for *F. hepatica* and *F. gigantica* are considered to be quite distinct, these flukes can adapt to various snail species provided other conditions are favourable. It was conclusively shown by Kendall (1950) that very young specimens of *L. stagnalis*, a typical aquatic snail, could be infected with *F. hepatica* and development could proceed to maturity. The Australian *L. tomentosa*, host of *F. hepatica*, was shown to be receptive to miracidia of *F. gigantica* from East Africa, Malaysia and Indonesia (Boray, 1966). Similarly, El Harith (1980) in his experimental study under laboratory conditions found that *F. hepatica* develops normally in *L. auricularia* as it does in *L. truncatula* and that *F. gigantica* develops normally in *L. truncatula* as well as in *L. auricularia* but the development was significantly influenced by temperature. Dinnik and Dinnik (1957) found *L. truncatula* susceptible to infection with *F. gigantica*, and found it to be the common snail host of *F. gigantica* in the irrigated area near the slopes of Mount Kenya. Therefore, the possibility of transmission of *Fasciola* spp. by alternative snail vectors should always be considered in those areas in which such snails occur with the more natural vector.

Larval stages of *Fasciola* spp. are true parasites of their snail hosts. They may cause severe pathology in the host producing acute, subacute and chronic disease states, which respectively, correspond to the early infection, developmental and accumulation phases in the development of the parthenitae (Boray, 1969). Each of these phases can result in snail mortality which, if significant, can influence the population of infected snails and hence the incidence and intensity of fasciolosis.

It has been shown that a number of biological agents in the snail's habitat have a modulating influence on the balance between *Fasciola* and its snail vectors, with possible epidemiological implications. For example, the larvae of trematodes belonging to the family Echinostomatidae, which also parasitise lymnaeids, feed on sporocysts and rediae of *Fasciola* spp. and other trematodes, thereby inhibiting the latter's larval development (Boray, 1969). Consequently, they are considered as potential biological control agents for *Fasciola* species. Similarly, *Chaetogaster limnaei*, an oligochaete commensal inhabiting the mantle cavity of several freshwater snails which catch and swallow miracidia and cercariae (Khalil, 1961), is potentially capable of influencing the intensity of fluke infection in snails and therefore in definitive hosts. In the Philippines, a field study by Dimatulac and Pinto (1983) showed that there was a significant negative correlation between the number of *C. limnaei* per snail and the occurrence of *Fasciola* larvae; an average of four *C. limnaei* per snail prevented the larval infection. In Kenya, Cheruiyot and Wamae (1989) and Wamae and Cheruiyot (1990) noted that the incidence of concurrent infections of *C. limnaei* and trematode larvae was very low and rise in number of snails infested with this commensal resulted in a decline in developmental stages of trematodes.

In a survey in Tanzania, Hammond (1965) found that quite often *L. natalensis* snails were infected with a trematode, xiphidiocercariae, and no double infections with larvae of *Fasciola* were seen. These finding led him to suggest that snails carrying an existing trematode infection may be unattractive to miracidia. Some species of planaria have also been reported to be antagonistic to the miracidium of *Fasciola* spp. (Tongson, 1978), however, neither the occurrence nor the epidemiological significance of these biological agent has been adequately evaluated.

2.6 Livestock Husbandry Practices and Systems of Production

The method of flock and herd management in any area has a major influence on whether or not the host comes into contact with infection as well as on the frequency and duration of that contact. There have been few relevant studies with regards to fasciolosis, therefore only general statements on its epidemiological significance based on the limited available information are possible.

In the tropics, there is abundant grazing and alternative sources of drinking water during the wet season. This reduces the need for animals to graze near, and to drink from, particular permanent water holes. Moreover, snail habitats and pastures are constantly flooded and therefore snails and free-living stages of *Fasciola* spp. are regularly flushed out and disseminated over large areas. In some cases animals are deliberately pastured away from wet marshy areas and permanent pools (Troncy, 1989). Even when snail habitats are localised, as in the case of *F. hepatica* endemic areas, animals would normally prefer to graze or browse on dry pasture and thereby avoid fluke infested parts of the pasture (Boray, 1969), as long as this choice of grazing or browse is available to them. Consequently, in most cases only light infections are likely to be acquired during the wet season. By contrast, as the rains cease and dry season sets in, there is a reduction of available grazing area and sources of drinking water for stock. This increases the need for animals to graze near and drink from permanent dams, water holes and other snail habitats which are the only sources of grazing and drinking water at that time of the year. The over-crowding resulting in increased contamination of these areas with fluke eggs soon gives rise to heavy infections in snails, a significant increase in metacercarial density on the herbage and disease outbreaks during the dry season and sometimes extending to the start of the succeeding rainy season, depending on the locality (Babalola and Schillhorn van Veen, 1976; Schillhorn van Veen, 1979; Troncy, 1989). In those parts of the tropics where the dry season lasts from the end of October to March or April, snail host densities start to increase around October, reaching a peak during November/December. This peak is maintained throughout the dry season (Troncy, 1989). A marked increase in herbage metacercarial density occurs by January and peak infestations during February/March. Consequently, outbreaks of acute fasciolosis are generally observed from late February and subacute and chronic disease a little later in the season and during the early part of the succeeding rainy season (Schillhorn van Veen, 1979; Troncy, 1989). A broadly similar pattern of infection has been described in Malawi by Mzembe and Chaudhry (1979, 1981) and in Nepal by Morel and Mahato (1987).

Migratory herds and flocks usually run relatively little risk of heavy infection except when they graze in or drink from heavily contaminated areas at some point in their migratory route. However, widespread heavy infection is one of the consequences of disruption of normal migratory patterns by prolonged drought and other natural disasters which result in migration to unusually distant and unfamiliar territories, prolonged stay and over-crowding at a few available water holes. These facts, coupled with malnutrition, climatic stress, concurrent infections and general lowering of body resistance were identified as being responsible for the unusually high incidence of helminthoses during the 1973 to 1974 drought in Northern Nigeria (Schillhorn van Veen, 1974). Transhumant flocks and herds also acquire significant infection from permanent snail habitats during the dry season but acquisition of infection ceases when they leave the permanent water holes at the end of the season (Troncy, 1989).

The risk of fasciolosis is greater in intensively managed sedentary, as opposed to migratory herds and flocks. However, this risk may be considerably reduced where a high standard of grazing management and other prophylactic measures are routinely applied. Such managerial practices as avoidance of permanent snail habitats, provision of alternative watering places during the dry season, use of low stocking rates, regular and strategic anthelmintic treatment of stock and, to some extent, various methods of snail control have a significant influence on the life-cycle of *Fasciola* and on the epidemiology of the disease (Sewell, 1976; Boray, 1982, Over, 1982). In some situations mixed grazing of sheep and cattle may also influence the incidence of fasciolosis, especially in the former more susceptible host, as the less susceptible host would ingest and destroy numerous metacercariae which would otherwise become available to sheep (Boray, 1969).

The increasing use of flood plains, irrigated land, manmade lakes and dams for livestock rearing has major implications for the spread and incidence of fasciolosis (Hammond, 1965; Schillhorn van Veen, 1979). In the permanently favourable environment existing in these areas infection would be present throughout the year and the optimum number of cercariae per annum would be expected to be produced in such places (Boray, 1982).

2.7 Modelling and Forecasting Fasciolosis

Two important meteorological factors in the development of *F. hepatica* are temperatures above 10°C and the presence of free water. In the late 1950's Ollerenshaw suggested that development is therefore usually impossible during the winter (too cold) and that there may be insufficient water during some of the summer months (too dry) (Ollerenshaw and Rowlands, 1959). This is the basis of the 'M_t' forecasting system for fasciolosis which was established in England and Wales in 1958 (Ollerenshaw and Rowlands, 1959; Ollerenshaw, 1966). M_t is a monthly index of wetness given by:

$$(R\text{-}p+5)n$$

where, on a monthly basis, R is the rainfall in inches, p is the potential transpiration and n is the number of raindays. Observations suggested that, since parasite development is also temperature-dependent, the rate of development is similar in June, July, August and September, but is halved in May and October, when the M_t index should therefore be halved. A seasonal M_t can be calculated by adding the M_t values for six-month period May to October. This sum simulates the progression of the disease in relation to changing meteorological conditions and can be used to predict if animals are at risk to fasciolosis, so that suitable prophylactic measures can be undertaken. The simple approach of this model enables its execution without a computer.

Ross (1970) in Northern Ireland suggested the use of the 'wet-day' as a suitable climate parameter on which to base an empirical approach to forecasting fasciolosis. By summating the number of wet-days each year from May until the end of September with predetermined values appropriate to a 'standard' year namely 12, 18, 36, and 48 wet-days it was possible to provide a continuing forecast throughout the summer. A similar association was noted in France by Ollerenshaw (1970). Later, this wet-day system was established for use in Northern Ireland (Ross, 1978) and France (Leimbacher, 1978). The wet-day system uses only the number of days in which more than 1 mm of rain fall is recorded, with the assumption that thermal factors are sufficiently uniform between years.

Later, an autoregressive, multivariate model was developed for use in Northern Ireland (Goodall, McIlroy, Stewart and McCracken, 1989). The national computerised information and retrieval system established in Northern Ireland to analyze specific condemnation data in abattoirs, integrates with a concurrent meteorological database which facilitates the quantification of the effect that individual weather variables have on the occurrence of fasciolosis. The computer system is used at the end of August to forecast the following year's prevalence which enables the formulation of an effective control strategy, based on the expected prevalence of the disease, to be implemented in September. McIlroy, Goodall, Stewart, Taylor and McCracken (1990) suggested that the forecasting system may be applicable for use in any region of world where relevant abattoir condemnation and meteorological data are available.

The climate forecast models have been adapted for use in the Southeastern USA, based on the growing degree day (GDD) concept and the Thornthwaite water budget (Malone, Williams, Muller, Geaghan and Loyacano, 1987). A microcomputer programme uses daily maximum and minimum temperature and daily rainfall data to calculate water budget values and a cumulative forecast index at any interval during the annual fluke transmission cycle, according to the following formula:

$$\left[\frac{S \times GDD_1}{C} + GDD_2\right]\frac{1}{D}$$

where S is daily surplus water, GDD_1 is degrees over a base value of 10°C (below which life cycle development ceases) and C is the number of days in the prior 14 days with mean temperatures of <10°C. GDD_2 is the same as GDD_1, but the values is not accumulated if soil moisture is absent in the top 2.5 cm. D is the number of days in the preceding 14 in which soil moisture storage was below the adequate level. C and D are adjustment factors to account for the effects of sustained periods of cold or drought, respectively. GDD_1 values are multiplied by daily surplus water to account for the influence of rainfall events on habitat flooding, release of cercariae from snail vectors and dispersal of infective stages onto surrounding pasture. It is assumed that vector snails, aestivate during dry periods and the transmission year ends as metacercariae are killed by sustained summer drought. In Louisiana, USA, the wet-day forecasting system correlated poorly with transmission data, whereas the M_t and Thornthwaite water budget-derived systems correctly ranked 5 of the 6 years in terms of annual numbers of flukes transmitted to sentinel calves (Malone, et al., 1987). Compared with the M_t system, however, the water-budget system more closely correlated with transmission data, on the basis of statistical regression analysis (Malone et al., 1987).

Recently, soil-hydrology geographic information system (GIS) models for estimating farm/site-specific risk of fasciolosis in cattle have been developed for use in the Chenier Plain and Red River basin ecologic zones of Louisiana (Zukowski, Hill, Jones and Malone, 1991). These models, which were developed by computer-based statistical and image analysis method using earth observation satellite data and soil-type maps, provide a practical approach for estimating the distribution of habitats of the intermediate host of *F. hepatica.* It was suggested that earth observation satellite data and soil maps can be used, with an existing climate forecast based on the Thornthwaite water budget, to develop a second generation model that accounts for both regional climate variation and site-specific differences in fasciolosis risk based on soil prone to snail habitat (Malone, Fehler, Loyacano and Zukowski, 1992).

Smith (1984a; 1987) described density-dependent constraints on population of *F. hepatica* that tend towards epidemiologic stability. He developed an analytical model of anticipated changes in the infection intensity of natural fluke population in sheep after single and multiple doses of different classes of anthelmintic (Smith, 1984b). Assuming a known cohort of metacercariae, the model allows hypothetical comparison of drug intervention within a given herd and environment, but not between herds.

Meek and Morris (1981) developed a more comprehensive computer simulation model for evaluating alternative control strategies for ovine fasciolosis at an experimental site in Australia. The model uses a combination of algebraic functions and Monte Carlo sampling from defined probability distributions and simulates the life cycle of *F. hepatica*, the population dynamics of *L. tomentosa*, soil moisture, pasture production, sheep feed intake and marketable products. Thus the model includes components to account for climate variation, snail habitat extent, metacercariae intake of sheep, and the effect of fluke burdens on productivity and economic return. The authors suggested that the model could be applied to other sites, but only after modifications that represented the new circumstances, notably snail habitat extent and stocking rate, the two variables that mostly influenced economic return.

Modelling has a broad remit, including the conceptual representation of any real event in mathematical terms. As described above, much of the literature on the modelling and forecasting of fasciolosis has been concerned with that caused by *F. hepatica*; virtually no model or computer expert system seems available for fasciolosis caused by *F. gigantica*. Computer expert systems can use a quantity and complexity of data that expand the ability of the mind to make sound judgements and they allow transfer of evaluation criteria to the novice that were gained by years of field experience. The computer models, which will use geographical information systems and environmental data bases, integrating the essential biology of three life histories- those of parasite, the snail intermediate host and the animal population at risk, and some means of evaluating the cost-benefit of control programmes, if developed in future, might be able to provide more realistic and practical approach for management of fasciolosis including for that caused by *F. gigantica*.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study Area

The study was conducted in the Koshi zone of eastern Nepal covering 5 Village Development Committee areas (VDCs) in the Terai (Sunsari and Morang districts) and 26 VDCs in the hills (Dhankuta, Terhathum and Sankhuwasabha districts) (Figs 3.1.1 and 3.1.2). The altitude in the study area ranged from 150 to 300 m in the Terai and 350 to 3500 m in the hills. From the agricultural point of view, the hills are generally refered to as low (below 1100 m), mid (between 1100 m and 1700 m) and high (between 1700 m and 2500 m) altitudes, and the area above 2500 m is known as high mountains. This classification of the altitude ranges has also been used in this study.

The climate in the Terai and lower hills is sub-tropical, and temperate in the middle and high hills. There are four defined seasons, namely spring (March-mid May), summer/monsoon (mid May-mid September), autumn (mid September-November) and winter (December-February). Rain occurs from May to September, maximum precipitation usually falling in July and August and an average monthly rainfall of between 400 to 600 mm during this period is not uncommon. December to March is usually very dry with little or no rainfall, often followed by pre-monsoon showers in April. In the hills, there are wide variations in temperature range depending on altitude and aspect of the location. Frost and misty conditions are characteristic of the high altitude area and frequent snowfalls occur during the winter. In the mid altitude hills, snowfalls are rare, however, frost occur many nights during the winter season. Owing to the more uniform topographical nature of the Terai, this area has a more homogeneous climate than the hills. The 15 years average (1977-1991) meteorological data recorded at the Pakhribas Agricultural Centre (mid altitude hills) and the Tarahara Agricultural Research Station (Terai) are shown in Appendix Table 3.1.

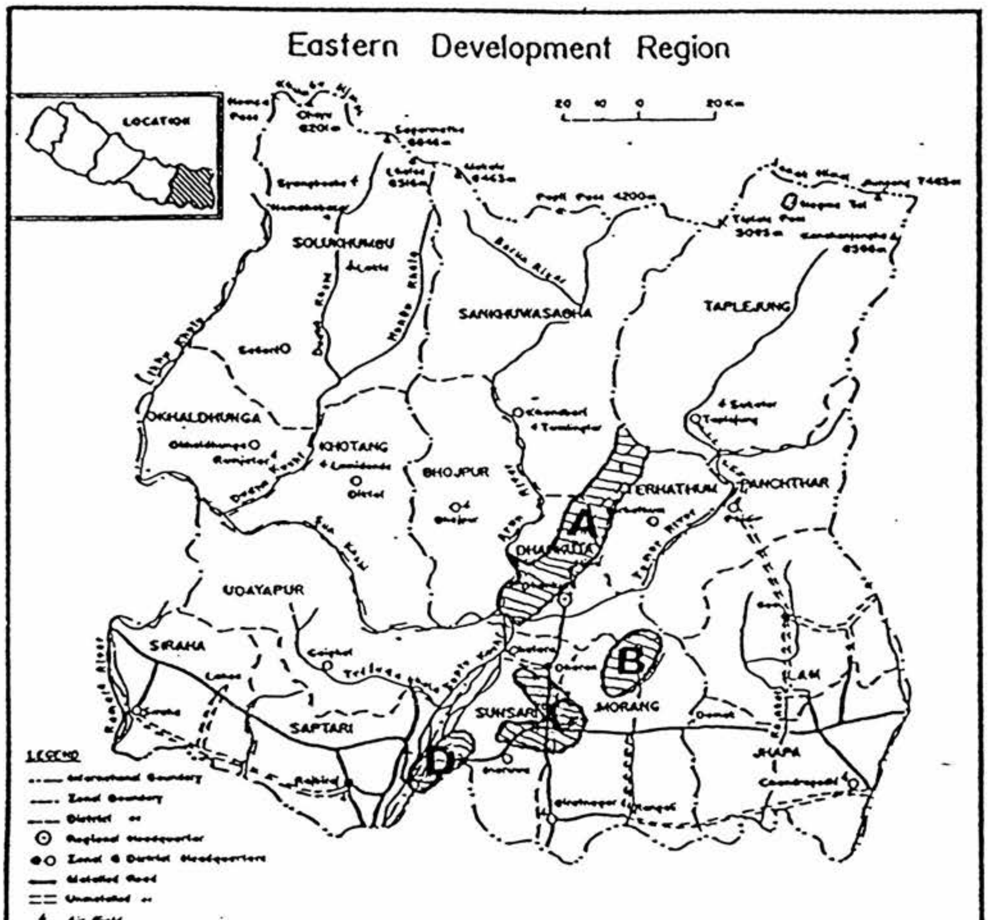


Figure 3.1.1. Map of eastern Nepal showing locations of the research sites in the hills (A) and the Terai: Letang (B), Tarahara, Itahari and Jhumka (C), Bhantabari (D).

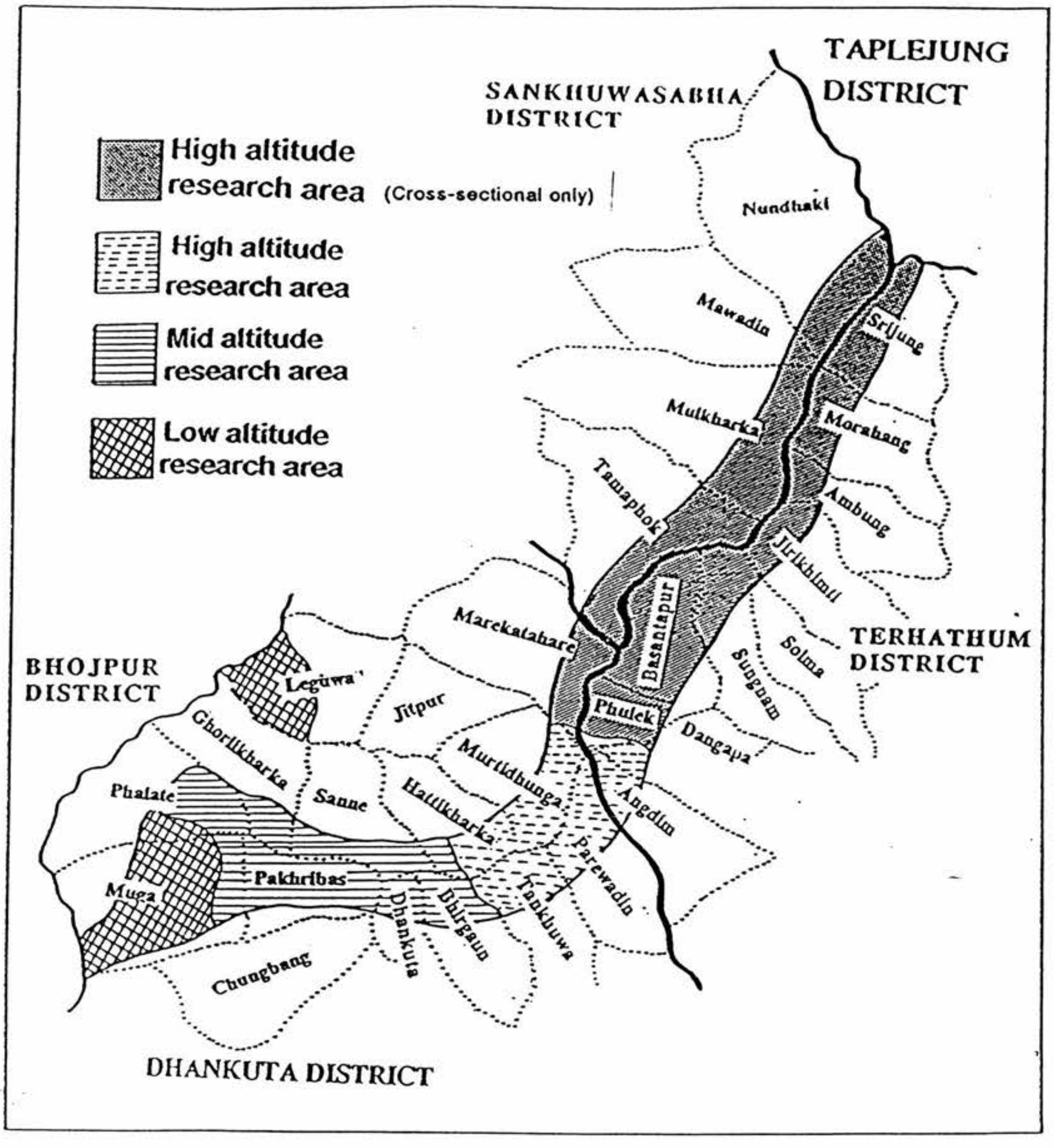
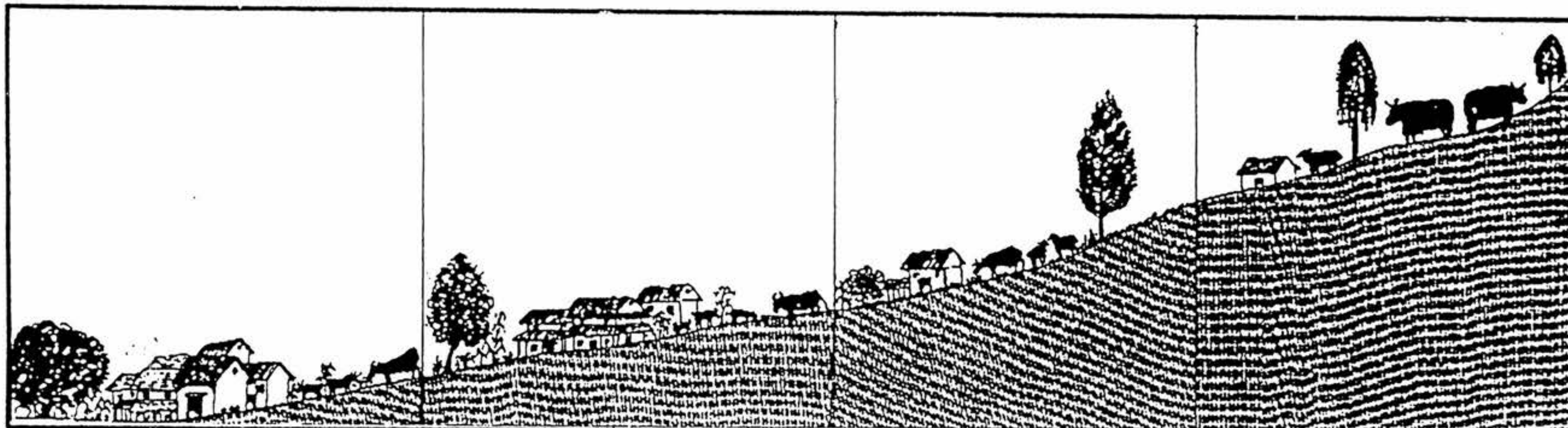


Figure 3.1.2. Map showing the low, mid and high altitude research sites in the hills.

The hill area is situated between two rivers, the Arun in the west and Tamur in the east; both the rivers have several tributaries originating from the study area. Most streams in the area are permanent. The river and stream valleys are narrow with fairly steep gradients creating local gullies especially towards the sources of the streams. The Terai area is mostly watered by the river Koshi through its many tributaries, notable among them are the Budhi and the Sunsari. Some of these tributaries flood to cover large areas in the monsoon creating wide continuous floodplains and shrink to small ponds or lakes in the dry season. Before a dam was built in the mid 1960s, the Koshi river changed its course frequently flooding the extensive adjacent areas. Besides many small local irrigation systems, a large irrigation scheme has recently been built to cover Morang and Sunsari districts through an extensive network of canals originating from the Koshi.

The livestock population in the study area is very dense and chiefly consists of cattle, buffaloes and goats. Sheep are kept in the middle and high hills but rarely on the Terai and lower hills. Almost all households keep one or more species of animals but the herds are small. The management systems vary widely from area to area and from farm to farm depending on species and breeding stage of the animals, climate, altitude, access to and ownership of certain classes of land, cropping pattern etc. However, the extensive method is most widely employed, i.e. the animals are grazed on fallow and marginal lands, communal pastures and river and road sides.

During the monsoon and subsequent flooding a large area of land in the Terai is utilised for cultivation of rice. Maize is grown in the higher areas and lighter soils. In the lower and middle hills, rice is grown in the irrigated and rain-fed *khet* (terraces with bonds to retain water) land. Wheat, mustard and lentils are major winter crops in the Terai and lower and middle hills. Potato and maize are the principal crops grown in the high hills. In the high mountains, no arable crop is grown. Transects of major farming systems are shown in Figure 3.1.3. The study sites covered almost all physiographic and agro-ecological zones of eastern Nepal, except for the yak and yakow (*chauri*) rearing area in the high mountains.



Terai and low altitude (90–1,100 m) Sub–tropical zone	**Mid altitude** (1,100–1,700 m) Warm temperate zone	**High altitude** (1,700–2,500 m) Cool temperate zone	**High mountain** (2,500–5,000 m) Alpine, Sub–alpine, Arctic zones
Crops: Rice–Rice, Wheat, Rice–Maize, Rice–Jute, Maize/Groundnut, Oilseeds; Chilli, Tomato, Brinjal etc. Mostly 2–3 crops per year	**Crops:** Rice–Wheat, Maize–Rice, Maize/Millet, Maize + Ground nut, Oilseeds; Cabbage, Peas, Leafy mustard Mostly one or two crops per year	**Crops:** Potato+Maize, Barley, Wheat; Radish, Beans Mostly one or two mixed crops per year	**Crops:** No arable crop grown; Upper limit of summer grazing
Livestock: Cattle, Buffaloes, Goats, Chicken, Pigs	**Livestock:** Cattle, Buffaloes, Goats, Chicken, Pigs	**Livestock:** Sheep, Goats, cattle, Buffaloes, Chicken	**Livestock:** Yak, Chauri, Sheep, Goats Mainly livestock enterprises
Forest and Fruits: Sal, Acacia plants, Ficus spp., Bauhinia spp.; Mango, Guava, Citrus, Sub–tropical fruits	**Forest and Fruits:** Pines, Utis, Nebaro, Katus, Chilaune, Khaneu; Plum, Peach, Pear	**Forest and Fruits:** Pines, Utis, Gogun; Apple, Pear, Plum, Peach, Walnut	**Forest and Fruits:** Conifer trees, Shrubs

Figure 3.1.3. Transects of inter-relationship between land use and altitude in eastern Nepal.

3.2 Snail Habitat Surveys

3.2.1 *Cross sectional survey*

The objective of this survey was to define the types of habitats available for and occupied by *Lymnaea* spp., the snail vectors of *Fasciola* spp. Some environmental factors were also investigated in an attempt to discover why some habitats should be more favourable than others.

The survey was carried out in 1991 during the dry season, January-February in the hills and March in the Terai when rain-water and floods had not obscured the main features of the habitats. Almost all the permanent water bodies èxisting in the research area were examined carefully for the presence of snails. If present, the snails were collected by hand and the number collected in 5 minutes was used as the quantitative criteria of the population density. The general features of the water bodies such as altitude, aspect, soil type, types of vegetation in and around the habitat, source of water, colour and turbidity of water were recorded. The readings of water pH, temperature, electro-conductivity (EC) and dissolved oxygen (DO_2) were taken respectively using electronic pH/Temperature (Model SE 105), Conductivity (Model SE 115) and DO_2 (Model SE 135) meters manufactured by Solex International, UK.

Approximately 10-15 representative snail specimens were preserved in 10% (v/v) formalin and brought to the laboratory at PAC for identification. The remaining snails were returned alive to the habitat.

3.2.2 *Longitudinal survey*

The snail ecology was studied for a period of 13 months from April 1991 to April 1992 by monthly visits to the representative selected habitats (26 habitats in the hills and 18 in the Terai) to record seasonal population fluctuations, snail breeding habits, i.e. egg masses and snail distribution. The approximate area of the habitats, and the pH, temperature, DO_2 and electro-conductivity of water in the habitats were also recorded.

Quantification of the population density was done by the method described above and expressed as per 5 man minute. Regardless of the size, up to 50 *Lymnaea* snails/shells were picked up from an area of the habitat. The collected snails were preserved either in 10% (v/v) formalin or 70% (v/v) ethanol and were brought to the laboratory at PAC for further examination. The population of dead (empty shells) and different sizes of snails were calculated on the basis of examination/measurements of the specimens in the laboratory.

3.3 Laboratory Based Experiment on the Ability of *L. auricularia* race *rufescens* and *L. viridis* to Survive in Drought Conditions

Although, this study was conducted in the laboratory at PAC, the experimental conditions were as natural as possible. The timing of the experiments were planned to coincide with the dry period between December and March 1992. Artificial habitats were made using 250 x 200 x 140 mm polypropylene trays. The mud collected from a natural snail habitat was placed to form a 30 mm thick layer on the trays. The mud was pressed, levelled and allowed to dry to semi solid consistency in the sun. Spring water was poured in the trays to about 15-20 mm deep above the surface of the mud. Thirty to 40 snails were placed in each tray, flaked fish food (Tropical fish food, Vetra Betta, Singapore) was provided and they were allowed to acclimatize to this environment for a week before the experiments were started.

The first experiment was started on the 18th December 1991 using 12 trays of mature snails, 6 each containing 18 to 20 mm long *L. auricularia* race *rufescens* and 6 containing 8 to 10 mm long *L. viridis*. Water was syphoned off from the trays except for the 4 trays used as controls, 2 containing each species of snails. When necessary, spring water was added and food was provided regularly to the snails in the control trays. All the cages were kept outdoors with an overhead shelter. On the 15th day, the surface soil samples from 2 dry trays containing each species were taken for moisture determination and then filled with spring water. The live snails were counted after 3-4 hours. The counting of the live snails was verified by examination of the crushed inactive snails under a stereomicroscope. The number of live snails in the control trays was also recorded. On the 30th day, the same procedures were repeated with the remaining trays of snails.

A similar design and procedure was used in the second experiment, except that an additional 6 trays of young (4-5 mm long) *L. auricularia* race *rufescens* were also included and all the trays were kept outdoors in the open, i.e. exposed to sunshine and weather conditions. The experiment commenced on 20th February 1992 and terminated after 30 days.

3.4 Farm/Flock Surveys

3.4.1 *Cross sectional survey*

A survey of all the households in the study area was conducted during January-February 1991 in the hills and during March 1991 in the Terai. Faecal samples were taken from all the cattle, buffaloes, sheep and goats, except for very young animals (less than 4-5 months old). Names of the owners and animals, along with age, sex, management system and history of anthelmintic treatments were recorded. The number and age of the animals of different species owned by the households were also recorded.

3.4.2 Longitudinal survey

To study the seasonal changes in the prevalence of chronic fasciolosis, the undrenched cattle, buffaloes and goats were randomly selected in proportion to the age and altitude of the total population. The sample size was set so as to give the true prevalence of the infection to within 10% at the 90% confidence level, assuming an average annual prevalence of about 35% (Cannon and Roe, 1982). This implies a sample size of 60 animals for each species. While these confidence limits were achieved for the total monthly prevalences both in the hills and the Terai, the sample sizes within the altitude ranges in the hills were not large enough to give this level of confidence.

To determine the incidence and seasonality of the infection, all the zebu calves (*Bos indicus*) (59 in the hills and 60 in the Terai) and buffalo calves (*Bubalus bubalis*) (35 in the hills and 58 in the Terai) under 2 years of age found negative for *Fasciola* spp. infection on faecal examination during the cross sectional survey, were selected. Each month faeces from individual calves was examined and the infection rate was recorded. The zebu calves found positive were treated with triclabendazole (Fasinex, Ciba Giegy) at the dose rate of 12 mg/kg, however, the positive buffalo calves were treated with oxyclosanide (Zanil, Imperial Chemical Industries Ltd) at the dose rate of 10 mg/kg, because triclabendazole is not effective against *F. gigantica* in buffaloes at the dose rate of 12 mg/kg which is recommended by the manufacturer (Mahato and Rai, 1992; Shrestha et al., 1992). These animals were again included in the study if negative on the three subsequent faecal examinations.

Fresh faeces were collected from the selected animals for 13 months on a monthly basis and brought to the laboratory at Pakhribas Agricultural Centre for examination. As the survey continued, so the total number of animals sampled per month declined due to sales or deaths. Also some of the owners resented the repeated sampling, however this problem was solved by providing them some basic veterinary drugs during each visit.

A questionnaire survey of the farming systems adopted by the owners of the selected animals was carried out each month. The survey included the collection of information relevant to the epidemiology of fasciolosis such as animal management and feeding practices, type and source of feed, grazing and watering places of the animals, type of crops grown by the farmer.

3.5 Slaughter Place Survey

Proper slaughter house and meat inspection practices do not exist in the study areas. There are, however, several *hat-bazaar* (weekly markets) where animals are slaughtered for the sale of meat. Budhabare and Hile *hat-bazaar*, which are situated in the hills 10 minutes and one hour walk respectively from the Pakhribas

Agricultural Centre, were selected. The survey was started in January 1991 and continued until July 1992. In the Terai, Letang *hat-bazaar* was selected and the survey was carried out between August 1991 and April 1992.

The livers of each animal slaughtered was superficially examined by lay personnel. Records were taken of age, sex, origin and management of the animals. One liver from Budhabare and 1 to 3 livers from Hile with intact gall bladders were purchased each week and brought to the PAC for detailed examination. Due to logistic problems, the slaughter place at Letang was visited on a monthly basis and the livers of slaughtered animals were examined only for the presence of flukes.

3.6 Laboratory Examinations

3.6.1 *Faecal examination*

A differential centrifugal flotation technique described by Sewell and Hammond (1972) was used. About 3 g of faeces were broken up and washed through a tea strainer with 42 ml of tap water. The well mixed suspension was transferred in to a 15 ml round-bottom, lipped, plastic centrifuge tube of 13 mm diameter and then centrifuged at about 1500 g for one minute. The supernatant was poured away and the sediment was resuspended in saturated salt solution and again centrifuged briefly. Again the supernatant was discarded and the sediment resuspended in zinc sulphate solution, of specific gravity 1.3. Carefully, zinc sulphate solution was added until the convex meniscus was slightly above the top of the tube. A short piece (c. 45 mm x 25 mm) of transparent adhesive tape (Sellotape, Sellotape Product Ltd., UK) was placed flat over the top of the tube and gently pressed on to the rim. After further centrifugation for three minutes the cellotape was lifted off, placed wet side down on a microscope slide and examined under the microscope at x100. The number of *Fasciola* eggs on the strip of tape were counted, multiplied by 1.3 (a factor which allows for the fact that some eggs are not recovered on the tape) and then expressed as eggs per gram of faeces (EPG).

3.6.2 *Liver examination*

The method of liver examination and recovering the flukes was that of Hammond (1970). Where the examination could not take place the same day, the liver was stored at 4° C. After weighing the liver, the gall bladder and all visible bile ducts were cut open and the flukes removed into physiological saline (0.85% w/v NaCl). The liver was then cut into 45 mm thick slices and squeezed, while fibrous areas were opened up to extract any further flukes. It was then cut into small cubes, which were squeezed again by hand before the debris was washed through a 4.75 cm into a 1.0 cm sieve, and the retained debris examined in aliquots in white trays so that any further flukes could be removed. If immature flukes were present, the cubes were squeezed in warm physiological saline and left, with periodic further squeezing, for about 4 hours at 37° C. They were then sieved, using first a 47.5 mm, then a 10 mm and finally a 0.5 mm sieve and aliquots were examined in petri dishes. In all cases the whole liver was examined. While counting the cut flukes, only the fragments which contained the ventral sucker were counted.

3.6.3 *Measurements and morphological study of flukes and eggs*

All flukes were measured unfixed and after death i.e. when they had lost all ability to contract. Those flukes which were recovered from fresh livers were kept at 4° C overnight before being measured. The flukes were gently smoothed out in a petri dish which then was placed on a graph paper (1 mm x 1 mm small squares) to measure the maximum length and maximum width of each fluke. It was most important that sufficient moisture was present in the petri dish and the flukes were never stretched.

Occasionally, eggs were obtained in physiological saline from the uteri of those flukes which had interesting morphological features by cutting a part of the body immediately posterior to the ventral sucker. At least 50 eggs from each such fluke were measured using a calibrated eye piece micrometer under a microscope at x400 magnification.

The morphological studies of the flukes were carried out on representative fixed and stained specimens. A fluke was gently smoothed out in a flat bottomed container and a microscope slide placed over it, another fluke was then placed on this microscope slide and another slide on the fluke. The process was continued until the "sandwich" was up to 20-25 mm high. Ten per cent formol saline was then poured gently into the container until the flukes were immersed. After 24 hours the slides were removed and the flukes transferred to 5% (v/v) formal saline. At the Centre for Tropical Veterinary Medicine (CTVM), the fixed and preserved specimens were stained with borax carmine and mounted by the usual method before their morphology was studied.

3.6.4 *Snail examination*

The species of the snails collected during the surveys were identified on the basis of their shell morphology using the criteria described by Hubendick (1951). Measurements of shell length were carried out under a stereomicroscope by aligning the specimens placed in a petridish with the lines of graph paper which were ruled in millimetres. However the length and width of the aperture (shell opening) were measured with a plastic ruler. The representative specimens were sent to Dr D S Brown, at the British Museum (Natural History), London for the confirmation of identification.

For detection of larval trematode infection, the snails were placed in a little water in a glass petri dish, the shell was crushed and then examined under stereomicroscope by teasing the internal organs with a fine needle. The *Fasciola* spp. and other trematode larvae were identified on the morphological basis as described by Frandsen and Christensen (1984) and Ollerenshaw and Graham (1986). The stages of *Fasciola* spp. infection were classified as immature (only rediae) and mature (rediae and cercariae).

3.7 Data Handling and Statistical Analysis

The data were collated and analyzed initially using a lap top computer (T1000 XE, Toshiba) and a spread sheet programme, Lotus 123, release 2.2 (Lotus Development Corporation). The graphics were created using the software SlideWrite Plus, version 4.10 (Advance Graphics Software, USA). The statistical packages MSTAT-C, version 1.3 (Michigan State University) and MINITAB, release 8 (Clecom Microcomputer Specialists, USA) were used for the statistical analysis.

To discover the differences in prevalence and incidence of infection in relation to locality, season, management, sex and age groups, a Chi-squared test was used. The non-parametric Kruskal-Wallis test was used to compare faecal egg counts of animals between different localities, season, management systems and age and, when appropriate, significance located using a Mann-Whitney test. The associations between snail densities and the physico-chemical characteristics of the waters and the altitude of habitats were assessed by calculating the Spearman's Rank Correlation Coefficients and their probabilities. In all analyses, the significance level was taken as $p < 0.05$. Further details of the data analysis are described with the relevant results.

CHAPTER FOUR

RESULTS

4.1 Meteorological Data

The monthly meteorological data recorded during the survey period at the Pakhribas Agricultural Centre (PAC), Dhankuta, sited at 1,667 m, latitude 27° 17', longitude 87° 17'in the hills and the Tarahara Agricultural Research Station (TARS), Sunsari, located at 200 m, latitude 26° 45', longitude 87° 25' in the Terai are presented in Appendix Table 4.1 and Figure 4.1.1. In general, these data were comparable with the previous 15 years average figures (Appendix Table 3.1) suggesting that the study period was a normal year.

At the PAC, the average minimum temperature varied between 4.4° C in January and 18.0° C in July while the average maximum ranged from 13.9° C in February to 26.1° C in April (1992). The total monthly rainfall varied from 0.0 mm to 469.9 mm and most of the rainfall occurred between May and September with an annual total of 1568.5 mm (April 1991 to March 1992). Except for November and March, wet days were recorded every month with the highest of 26 days in July and August, the annual total being 122 days (April 1991 to March 1992). The lowest relative humidity (67%) was recorded in April 1991 while the highest (93%) was recorded in September.

Except for the relative humidity, the climatological features recorded at the TARS were more or less similar to those at the PAC, although there were some differences in the actual values. The lowest minimum temperature was 10.7° C in January and the highest maximum temperature reached up to 32.9° C in April 1991 and 35.2° C in April 1992. Although there were less wet days (annual total 98 days), the total annual rainfall (2089.9 mm) was higher than at the PAC. At the TARS, the relative humidity varied between 56% in April 1992 and 95% in January. However, unlike the PAC, the relative humidities were higher during December-February than those during July-September.

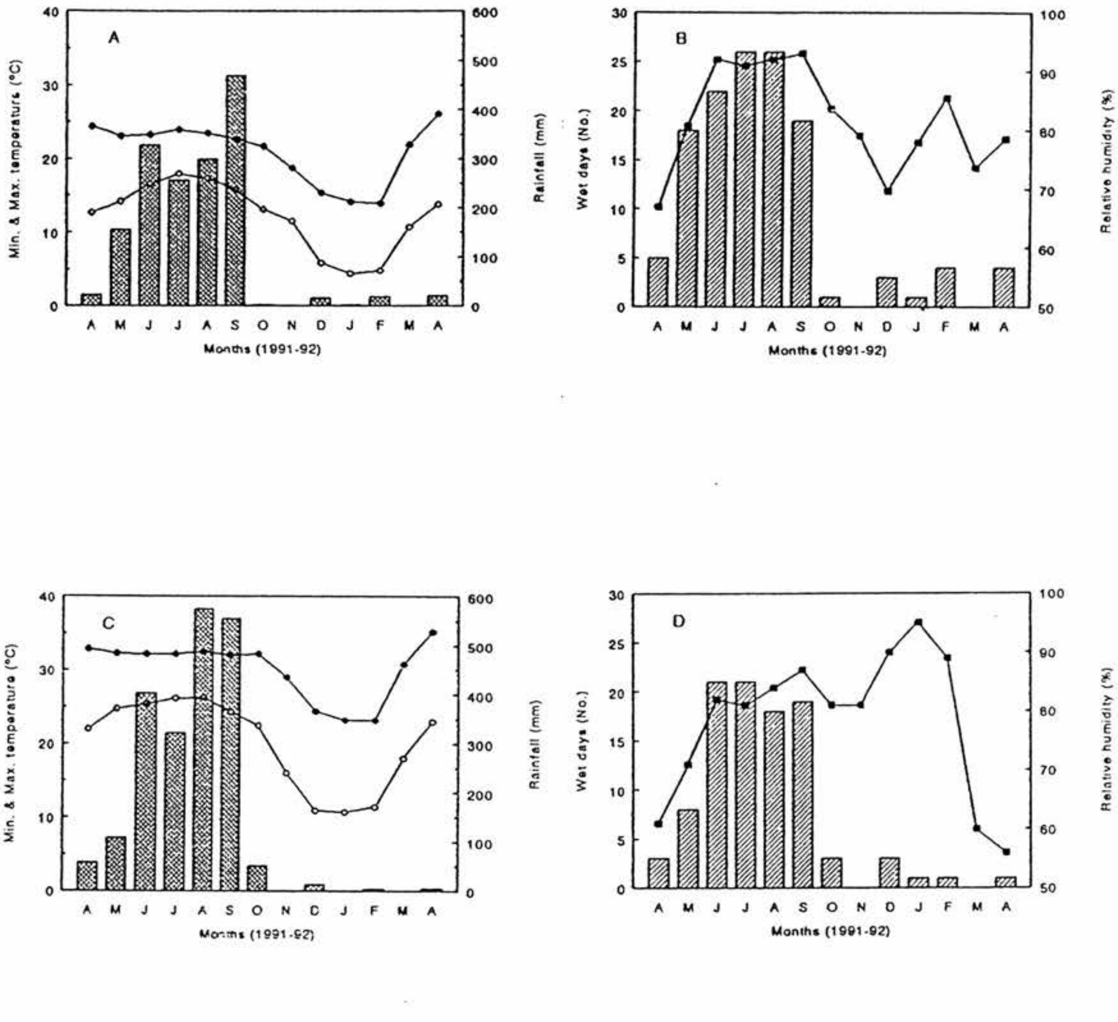


Figure 4.1.1. Meteorological data recorded at the Pakhribas Agricultural Centre, Dhankuta in the hills (A and B) and Tarahara Agricultural Research Station, Sunsari in the Terai (C and D) during the study period (1991-92): monthly average minimum (O) and maximum (●) temperatures, total rainfall (▩), number of wet days (▨) and relative humidity (■).

4.2 Snail Habitat Surveys Data

Raw data for the cross-sectional and longitudinal surveys of snail habitats are recorded in Appendix Tables 4.2.1-4.2.4.

4.2.1 *Species of Lymnaea snails*

The majority of snails were easily separated into three types at the time of collection namely *Lymnaea* spp., Planorbid spp. and Bithyniid spp. The main problem of identification concerned the speciation of *Lymnaea* snails. As often occurs with snail identification the specimens showed some variation in shell shape and most appeared intermediate between two species on the basis of descriptions given by Hubendick (1951). An appraisal of all the characters, however, suggested that the 1405 specimens examined belonged to either of the four *Lymnaea* spp. namely *L. auricularia* race *rufescens* Gray more often known as *L. acuminata* Lamarck, *L. auricularia sensu stricto*, *L. luteola* Lamarck and *L. viridis* Quoy and Gaimard (Figure 4.2.1).

These identifications were also confirmed by Dr D S Brown, British Museum (Natural History) to whom 150 representative specimens from 13 habitats were sent. However, he considered that the specimens identified as *L. viridis* could be *L. andersoniana* Nevill.

L. auricularia race *rufescens* was found to be the predominant species which was widely distributed in the Terai as well as at the low, mid and high altitudes in the hills, while *L. auricularia sensu stricto* were found only at the mid and high altitudes in the hills (Appendix Tables 4.2.1 and 4.2.2). *L. luteola* were commonly present in the Terai and at the low and mid altitudes, whereas the distribution of *L. viridis*/*L. andersoniana* was restricted to the mid altitudes.

Figure 4.2.1. Shells of *L. auricularia sensu stricto* (top left pair), *L. luteola* (top right pair), *L. viridis* (bottom left pair) and *L. auricularia* race *rufescens* (bottom right pair).

4.2.2 *Habitat types available for and occupied by Lymnaea spp.*

Of the 256 sampling sites (187 in the hills and 69 in the Terai) which were investigated, a total of 122 (85 in the hills and 37 in the Terai) harboured either one or more *Lymnaea* species. The types of habitats which were available for and occupied by these snails in the study area are summarised in Table 4.2.1.

Table 4.2.1. Summary of habitat types available for and occupied by *Lymnaea* spp.

Area	Habitat types	Habitats examined (No)	Presence of *Lymnaea* spp. (No. of habitats)			
			Abundant* (> 100)	Moderate* (10-100)	Scanty* (< 10)	Absent
Hills	1. Spring fed ricefields	65	12	28	22	3
	2. Stream fed ricefields	14	5	3	4	2
	3. Irrigation channels	2	1	0	1	0
	4. Springs	73	0	1	4	68
	5. Streams	9	0	0	0	9
	6. Seepage from springs/streams	5	0	0	3	2
	7. Pools fed by springs, streams, rain-water etc.	17	0	0	1	16
	8. Rivers	2	0	0	0	2
Terai	1. Ponds	24	2	6	5	11
	2. Road-side pools	23	0	7	6	10
	3. Irrigation canals/channels	13	1	3	0	9
	4. Seepage from canals	4	0	3	0	1
	5. Ditches around drinking water place	3	3	0	0	0
	6. Rivers	2	0	0	1	1

* Figures in parentheses indicate snail density expressed as counts per 5 man minute.

In the hills, there were numerous springs resembling marshy type seepage. Near settlements, either people had dug a small pool to collect the water from the springs or the water was channelled to a tap for domestic use. In the high altitudes, most of these springs formed headwaters of streams. In the middle and low altitude areas, however, the land below springs was generally terraced for rice cultivation. Also, some ricefields were fed by water through irrigation channels. Although the major part of these fields were dry during the survey period (January-February), a few terraces near the springs contained water (Figure 4.2.2). These permanently wet terraces of the ricefields were found to be the main habitats of *Lymnaea* spp. in the hills. Streams themselves do not appeared be habitats of *Lymnaea* spp., possibly because conditions in them vary greatly in relation to rainfall, so that they may be rushing torrents or almost entirely dry.

Figure 4.2.2. Snail habitats in the hills: spring fed ricefields.

In the Terai, there are numerous ponds which have been constructed for the purpose of fish farming. Similarly, pools formed in borrow-pits created by the extraction of roadbuilding materials were a familiar sight. These ponds and pools (Figure 4.2.3) were found to be the major permanent snail habitats. The Koshi and Budhi rivers themselves were unsuitable for *Lymnaea* spp., possibly because of their fast current and heavy silt loads. However, *Lymnaea* spp. were found to flourish in the extensive irrigation systems and also in the seepages which are associated with the systems.

4.2.3 *Influence of environmental factors on the distribution of Lymnaea spp.*

The habitats with clean and slow running water appeared to be more suitable for *Lymnaea* spp. than those with turbid and stagnant water. The snails were almost invariably absent in the rivers or streams in which currents were very fast. Similarly, no *Lymnaea* spp. were seen in small wallowing pools for buffalo (Figure 4.2.4) and in water bodies in which either algae or some macrophytic vegetation were absent. A feature of many water bodies where *Lymnaea* spp. were absent was the occurrence of a heavy red flocculent precipitate on the bottom (Figure 4.2.4). This was very common in seepages, but was also quite frequent in roadside pools and ricefields.

The distribution of *Lymnaea* habitats in relation to soil type is shown in Table 4.2.2. As can be seen from the table the water bodies which had clay or loamy soil bottoms were more favourable habitats than those with sandy soil. Almost all the water bodies in sandy soil including a single cement tank which were positive for *Lymnaea* spp. had either a thin layer of mud or algae on the bottom.

Table 4.2.2. Distribution of *Lymnaea* habitats in relation to under water soil type.

Soil type	Hills			Terai		
	Total water bodies	No.+ve for *Lymnaea* spp	% +ve for *Lymnaea* spp	Total water bodies	No.+ve for *Lymnaea* spp	% +ve for *Lymnaea* spp
Clay	62	29	46.8	29	17	58.6
Loam	92	51	55.4	32	18	43.8
Sand	32	5	15.2	7	1	14.3
Rock	1	0	0.0	0	-	-
Cement	0	-	-	1	1	100.0

Figure 4.2.3. Snail habitats in the Terai: a fish pond (top) and a road side pool (bottom).

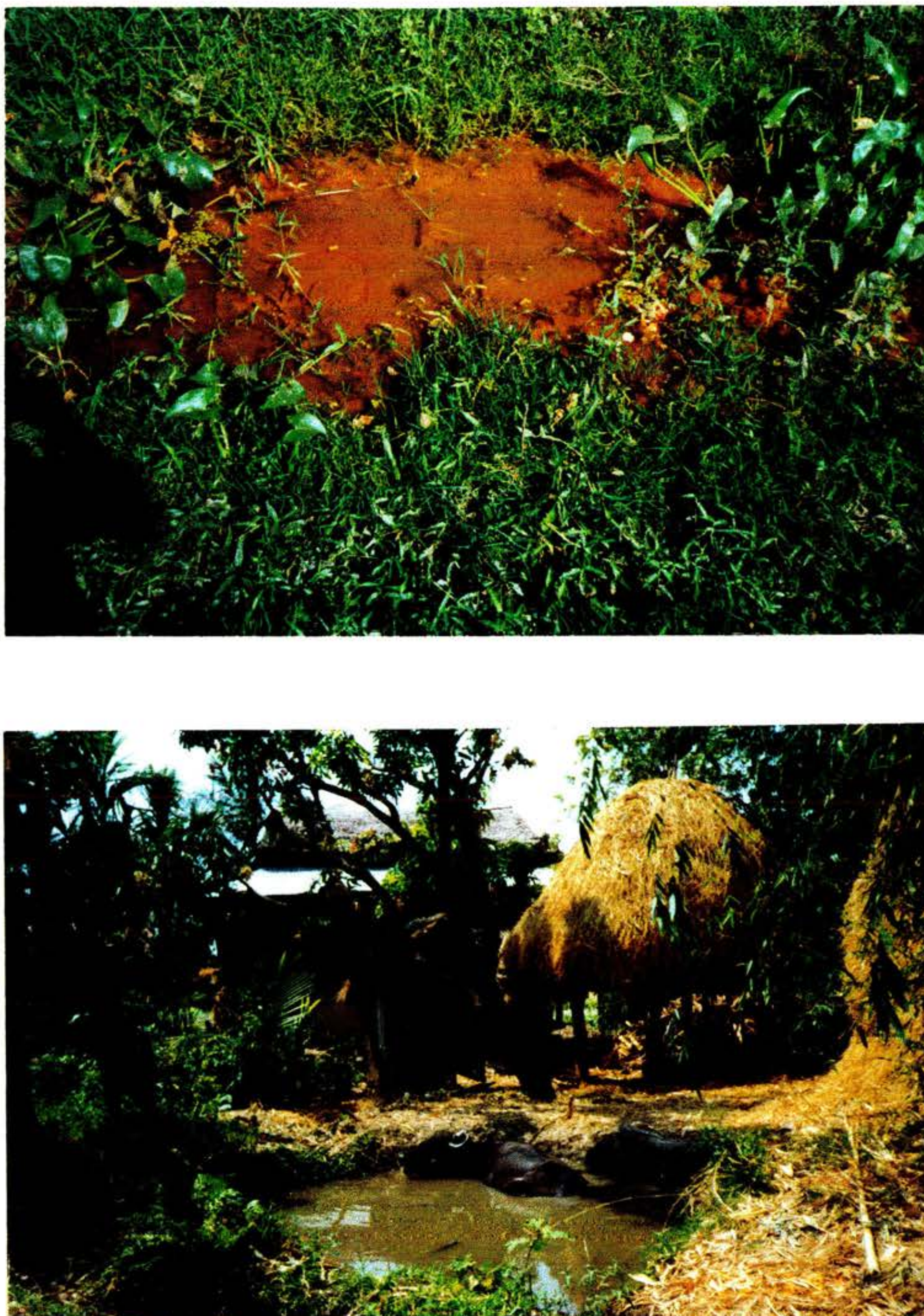


Figure 4.2.4. Water bodies which were unsuitable as *Lymnaea* habitats: a stagnant marshy ditch with red flocculent precipitates on the bottom (top) and a buffalo wallowing pool (bottom).

The vertical distribution of the water bodies as well as that of the habitats which were positive for *Lymnaea* spp. was more abundant between the altitude range of 1000-1750 m (Figure 4.2.5). The highest altitude at which the snails were found was 2100 m. The Spearman's rank correlation coefficient indicated that the percentage of water bodies harbouring Lymneid snails was negatively correlated with altitude ranges (Table 4.2.3).

Table 4.2.3. Spearman's rank correlation coefficients (r_s) for the distribution of *Lymnaea spp.* (% of habitats with *Lymnaea* spp.) against altitude, temperature, pH, electro-conductivity and dissolved oxygen.

Environmental factors	Hills			Terai		
	r_s	d.f.	P	r_s	d.f.	P
Altitude	-0.8530	11	<0.001	-	-	-
Water temperature	0.9636	8	<0.001	-0.3214	5	0.482
Water pH	0.9286	6	0.001	0.1061	8	0.771
Electro-conductivity	0.0333 (0.8929)*	7 (5)*	0.932 (0.007)*	-0.3584 -	10 -	0.253 -
Dissolved oxygen	0.5238	6	0.183	-0.0955	9	0.780

* The values which were obtained when the two odd observations (from the upper extreme of data) were omitted from the analysis.

The water temperatures which were recorded between 0800 and 1200 hours varied from 6.0 to 32.5°C (during Jan-Feb) in the hills and from 20.1 to 37.3°C (during March) in the Terai (Figure 4.2.6). In the hills, there was a significant positive correlation between the percentage of water bodies with Lymneid snails and water temperature, however, this was not the case in the Terai (Table 4.2.3).

The snails were found in water bodies in which water pH ranged from 5.1 to 9.0 in the hills and from 5.1 to 9.5 in the Terai, however, the highest frequency of positive habitats were within a pH range of 6.6-7.0 in the hills and 7.1-7.5 in the Terai (Figure 4.2.7). In the hills, the percentage of water bodies positive for *Lymnaea* spp. was positively correlated with the water pH, however, no correlation was found between these variables in the Terai (Table 4.2.3).

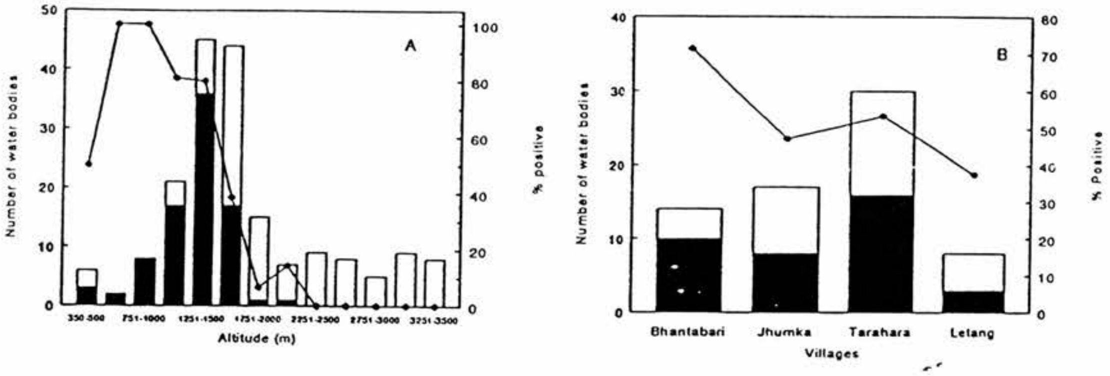


Figure 4.2.5. Distribution of *Lymnaea* habitats at the different altitude ranges in the hills (A) and in 4 villages in the Terai (B): number of water bodies positive for *Lymnaea* spp. (■), number of water bodies negative (□) and the percentage of water bodies positive (●).

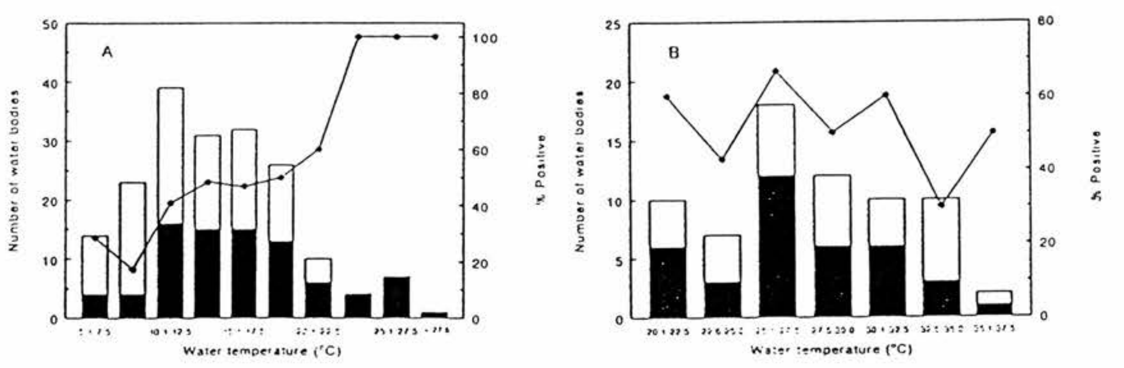


Figure 4.2.6. Distribution of *Lymnaea* habitats in relation to water temperature in the hills (A) and the Terai (B): number of water bodies positive for *Lymnaea* spp. (■), number of water bodies negative (□) and the percentage of water bodies positive (●).

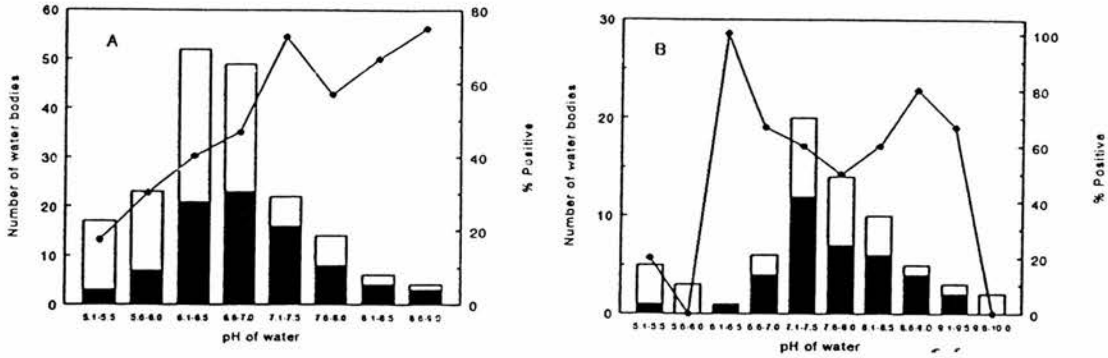


Figure 4.2.7. Distribution of *Lymnaea* habitats in relation to water pH in the hills (A) and in the Terai (B): number of water bodies positive for *Lymnaea* spp. (■), number of water bodies negative (□) and the percentage of water bodies positive (●).

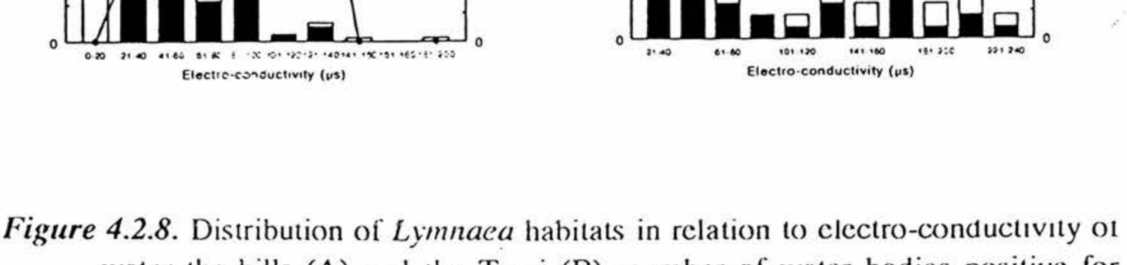


Figure 4.2.8. Distribution of *Lymnaea* habitats in relation to electro-conductivity of water the hills (A) and the Terai (B): number of water bodies positive for *Lymnaea* spp. (■), number of water bodies negative (□) and the percentage of water bodies positive (●).

In the hills, the electro-conductivity of the water ranged from 6.9 to 199.6 μs. However, these values were restricted between 21.0-140.0 μs with the highest frequency at the range of 41.0-60.0 μs in those water bodies which were positive for *Lymnaea* snails (Figure 4.2.8). In the Terai, the upper range of electro-conductivity of the water was found to be up to 240.0 μs, however, most of the positive habitats contained water with electro-conductivity ranging from 21.0 to 60.0 μs. There was no correlation between the percentage of habitats positive for *Lymnaea* spp. and the electro-conductivity of the water (Table 4.2.3). However, a significant positive correlation between these variables was found in the hills when the two odd observations from the upper extreme (above 140 μs) were omited from the analysis.

The number of the total water bodies investigated, the number of water bodies positive for *Lymnaea* spp. and the percentage of positive water bodies in relation to the dissolved oxygen concentration in water are shown in Figure 4.2.9. The dissolved oxygen concentrations varied from 0.1 to 6.5 mg/l in the hills and from 0.9 to 10.9 mg/l in the Terai. There was no correlation between percentage of the positive water bodies and the dissolved oxygen cocentrations in water (Table 4.2.3).

4.2.4 *Influence of environmental factors on the population density of* Lymnaea *spp.*

The pooled data from the hills and the Terai for the population densities ($\log_{10}$+1 counts per 5 man minute) of *Lymnaea* snails in relation to altitude, water temperature, water pH, electro-conductivity of water and dissolved oxygen in water are shown as scatter plots in Figures 4.2.10 and 4.2.11. Although, the highest population density was recorded at an altitude of about 300 m, densely populated habitats were found up to around 1600 m. Water temperatures up to 35°C do not appear to affect the density, however, the higher densities were mainly observed between 22-25°C. Although, a few habitats with high snail density had very high water pH ranging between 8.5 and 9.3, in most of the densely populated habitats this ranged between 6.0 to 8.0; below this pH range, snail density was generally sparse.

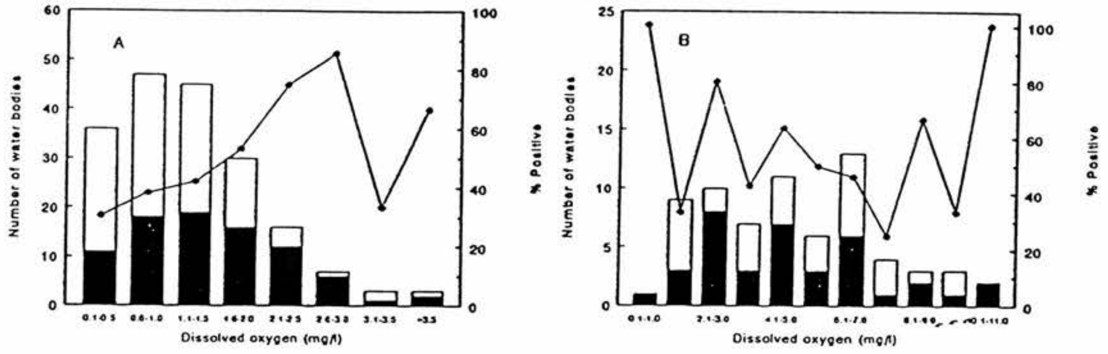


Figure 4.2.9. Distribution of ***Lymnaea*** habitats in relation to dissolved oxygen in water in the hills (A) and in the Terai (B): number of water bodies positive for *Lymnaea* spp. (■), number of water bodies negative (□) and the percentage of water bodies positive (●).

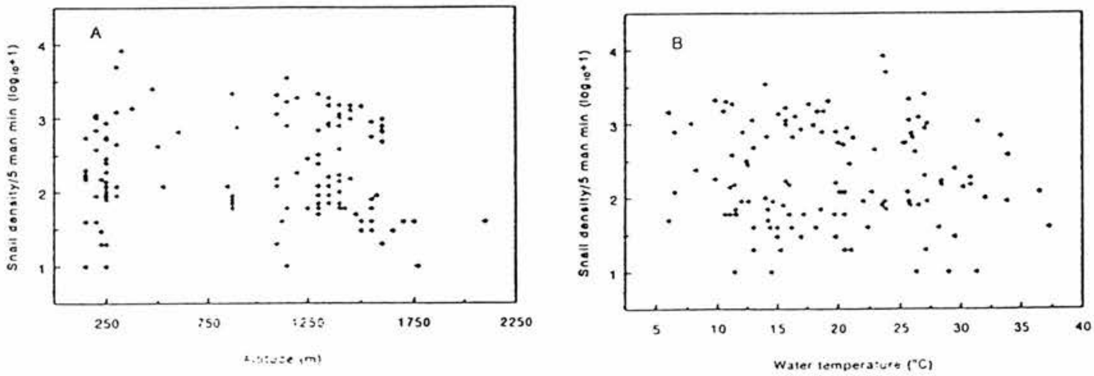


Figure 4.2.10. Scatter plot of snail density against altitude (A) and water temperature (B) of the habitats in the hills and the Terai.

There was no definite pattern of the population density in the habitats with the electro-conductivity of water ranging between 20 and 100 μs. Above this range, however, most of the habitats had higher snail densities which appeared to increase with the increase in electro-conductivity. Only comparatively few *Lymnaea* habitats had dissolved oxygen concentration greater than 3.0 mg/m but these were all densely populated (Figure 4.2.11).

The Spearman's rank correlation coefficients against each other of the population density, altitude, water temperature, water pH, electro-conductivity of water and dissolved oxygen concentration in water are given in Table 4.2.4.

Table 4.2.4. Spearman's rank correlation coefficients (r_s) together with their significance (P) at 120 d.f. against each other of the population density ($\log_{10}$+1 counts/5 man minute) of *Lymnaea* spp., altitude, water temperature, water pH, electro-conductivity of water and dissolved oxygen in water.

Environmental factors	Altitude of habitats		pH of water in the habitats		Temperature of water		Electro-conductivity (EC)		Dissolved oxygen (DO_2)	
	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P
pH	-0.643	<0.001	-	-	-	-	-	-	-	-
Temperature	-0.680	<0.001	0.398	<0.001	-	-	-	-	-	-
EC	-0.346	<0.001	0.133	0.144	0.315	<0.001	-	-	-	-
DO_2	-0.644	<0.001	0.475	<0.001	0.444	<0.001	0.159	0.080	-	-
Density of snails	-0.042	0.656	0.124	0.174	-0.048	0.599	0.215	0.017	-0.045	0.623

The snail population density was correlated only with the electro-conductivity of water. Except for the snail density, all of these variables were negatively correlated with altitude. The pH of water was positively correlated with the water temperature and dissolved oxygen, while the electro-conductivity and dissolved oxygen were positively correlated with the water temperature.

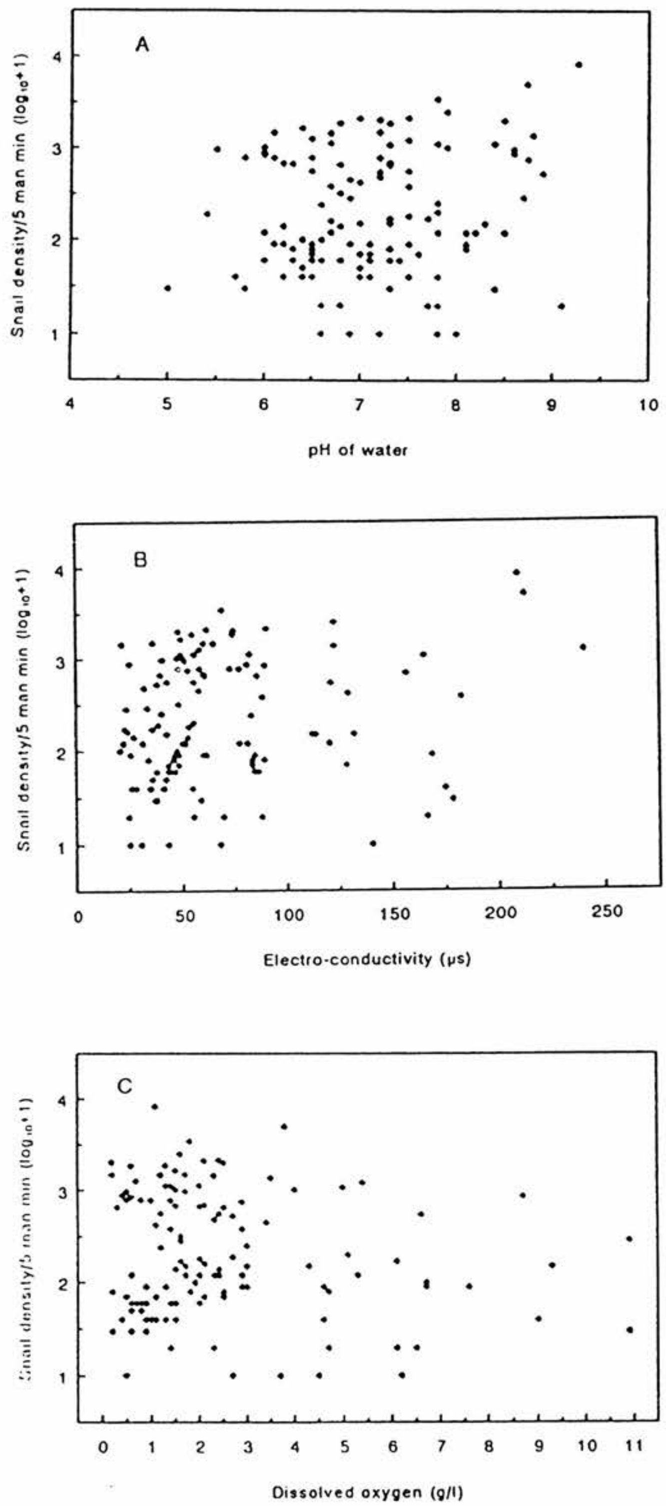


Figure 4.2.11. Scatter plot of snail density against water pH (A), electro-conductivity of water (B) and dissolved oxygen concentration in water (C) in the habitats in the hills and the Terai.

4.2.5 *Seasonal changes in the environment of habitats*

The habitats under study were flushed after heavy rainfall and flooded with running water during the monsoon. Habitat size was enormously increased during this period (Figure 4.2.12). In the Terai, many permanent habitats were connected to the extensive areas of inter-connected ricefields by running water. Most of these ricefields dried out completely in December and remained dry until mid May. In the hills, however, the areas of terraced ricefields near springs contained water throughout the year, thus serving permanent snail habitats.

The mean water temperature of *Lymnaea* habitats ranged from 14.7°C in February to 25.2°C in July in the hills and from 19.9°C in December to 34.2°C in July in the Terai (Figure 4.2.12). The monthly variation in water temperature was extremely significant (F_{12} = 9.58, P = <0.0001 in the hills and F_{12} = 21.69, P = <0.0001 in the Terai). Significantly lower mean temperatures (t_{24} = 2.27, P = 0.0324), ranging from 14.5°C to 21.5°C were recorded in the habitats which were negative for the snails in the hills (Appendix Table 4.2.5). In the Terai, however, differences between the mean water temperatures in the positive and negative habitats (Appendix Table 4.2.6) were not significant (t_{24} = 0.39, P = 0.6966).

The mean water pH in the positive habitats varied between 6.7 and 7.3 in the hills and 7.0 and 9.8 in the Terai (Figure 4.2.12). There was no significant difference in the seasonal changes in water pH in the hills (F_{12} = 1.80, P = 0.0514), while in the Terai, the values which were lower during the wet months, significantly increased as soon as the dry season started (F_{12} = 13.32, P = <0.0001). Differences between the mean water pH in the negative and positive habitats (Appendix Tables 4.2.5 and 4.2.6) were not significant (t_{24} = 0.61, P = 0.5457 in the hills and t_{24} = 0.23, P = 0.8182).

In the hills, generally the monthly mean values for electro-conductivity of water in the positive habitats were higher in the wet months, while this was not the case in the Terai as the highest value was recorded during December (Figure 4.2.12 and Appendix Tables 4.2.5 and 4.2.6). The monthly variation in these values was significant in the hills (F_{12} = 3.04, P = 0.0006) but not in the Terai (F_{12} = 1.20, P = 0.2926). Similarly, there was significant difference between the mean electro-conductivity of water in the positive and negative habitats in the hills (t_{24} = 3.40, P = 0.0024) but not in the Terai (t_{24} = 0.00, P = 1.0000).

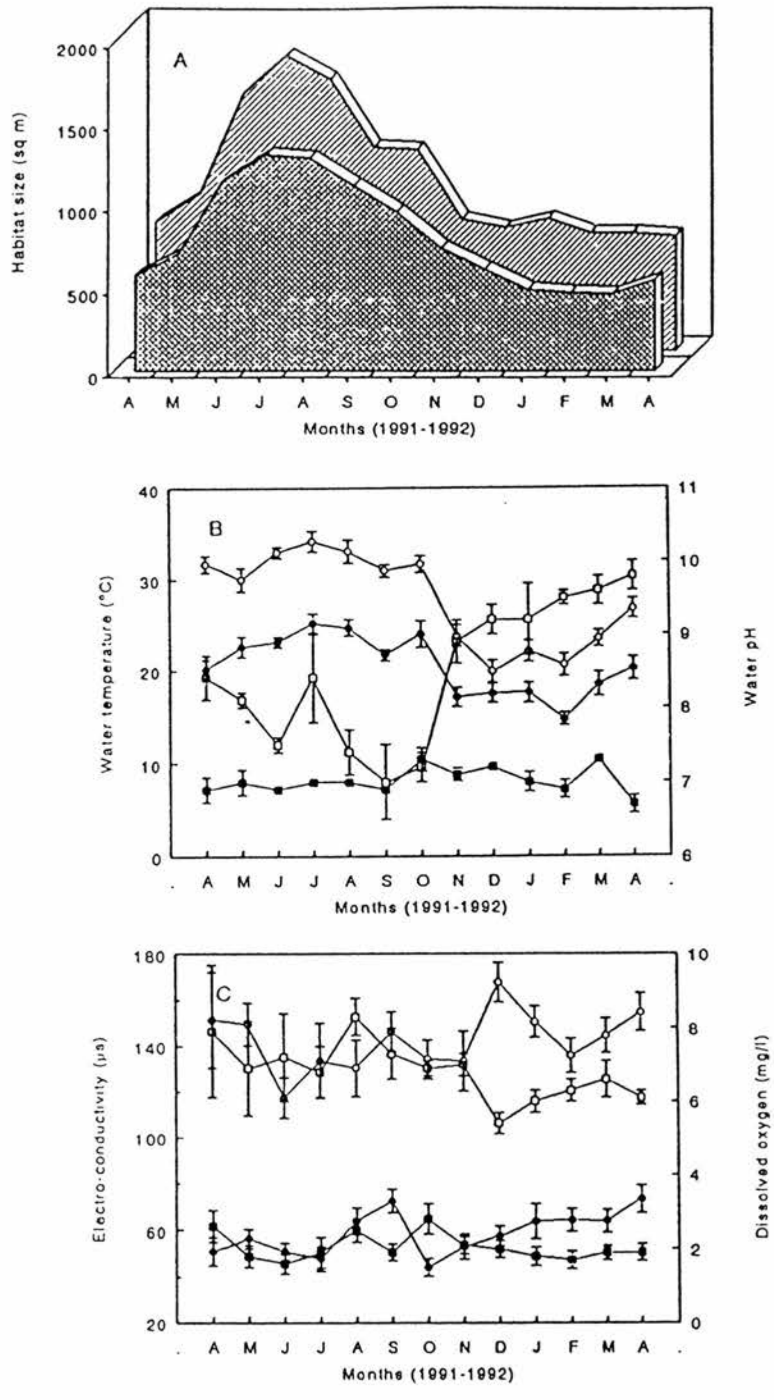


Figure 4.2.12. Seasonal changes in environments of the *Lymnaea* habitats: (A) approximate mean size of the habitats investigated (▩) and of those which were positive for the snails (▨); (B) mean ± SE water temperature in the hills (●) and the terai (○), water pH in the hills (■) and the Terai (□); (C) electro-conductivity in the hills (●) and the Terai (○) and dissolved oxygen in the hills (■) and the Terai (□).

The monthly mean values for dissolved oxygen concentration in water in the positive habitats ranged from 1.6 mg to 2.8 mg in the hills and 5.4 mg to 8.3 mg in the Terai (Figure 4.2.12 and Appendix Tables 4.2.5 and 4.2.6). Although, the values were lower during December-April (1992) than the other months, the variation was not significant either in the hills (F_{12} = 1.48, P = 0.1344) or in the Terai (F_{12} = 0.78, P = 0.6708). Also, there was no significant difference between the mean values in the positive and negative habitats (t_{24} = 1.16, P = 0.2573 in the hills and t_{24} = 0.78, P = 0.4405 in the Terai).

4.2.6 *Seasonal changes in the population density of Lymnaea spp.*

The seasonal changes in population density of *Lymnaea* spp. are summarised in Appendix Tables 4.2.7 (hills) and 4.2.8 (Terai) and illustrated in Figure 4.2.13.

In the hills, snail egg masses were more abundant during the dry season; there was a sharp fall in the mean population score during May-June, fluctuating at low levels until November and a sharp rise thereafter to reach a peak in January. After a slight decline in February, again the score reached a second peak in April. The Kruskal-Wallis test indicated that the monthly variation in the scores for the egg mass population was not significant (H_{12} = 12.57, P = 0.402). Similarly, higher concentrations of young *Lymnaea* spp. (length < 5 mm) were found between December and April than the other months, however, the monthly variation was not significant (H_{12} = 11.62, P = 0.477). The population density of those snails which were 5 mm or longer in size, declined from May to September and gradually increased thereafter, reaching a peak in April. The monthly variation in the population density was significant (H_{12} = 26.05, P = 0.011).

In the Terai, the seasonal pattern of the egg mass population was similar to that in the hills, however, the monthly mean scores were higher with a significant variation during the different months (H_{12} = 24.88, P = 0.016). Young *Lymnaea* snails (shell length < 5 mm) were found almost every month with the peaks in April (1991) and February. The concentrations of young snails were lower during August-November than the other months and the monthly variation was highly significant (H_{12} = 32.56, P = 0.001). Seasonal changes in the population of those snails which were either 5 mm or

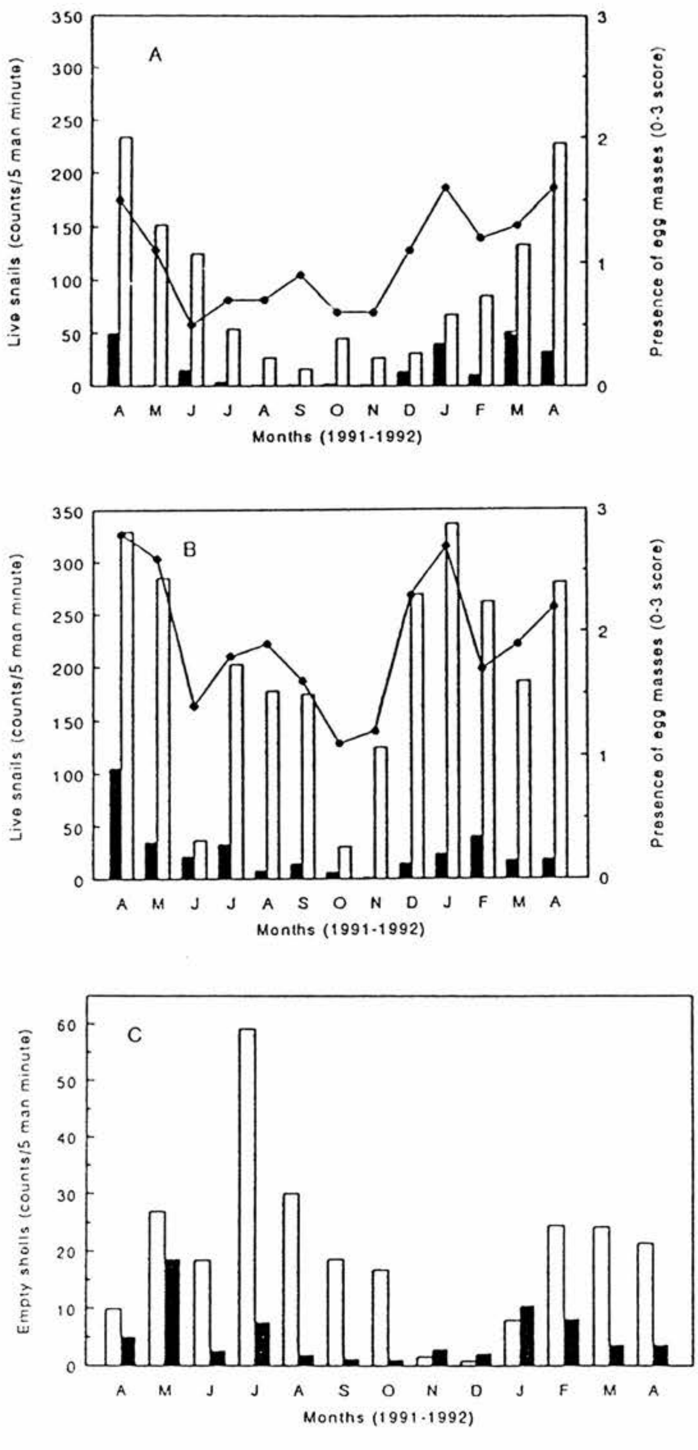


Figure 4.2.13. Seasonal changes in the mean snail counts: (A) live snails less than 5 mm (■) and 5 mm or longer (□), egg masses (●) in the hills; (B) live snails less than 5 mm (■) and 5 mm or longer (□) and egg masses (●) in the Terai; (C) empty shells in the hills (■) and the Terai (□).

longer in size, followed a pattern which was almost similar to that of the egg masses. However, the monthly variation in the population was not significant (H_{12} = 11.59, P = 0.480).

While the highest density of dead snails (empty shells) was observed in May in the hills, it was at its peak in July in the Terai. The monthly variations were significant in the hills (H_{12} = 23.31, P = 0.026) as well as in the Terai (H_{12} = 23.50, P = 0.025).

4.2.7 *Prevalence of Fasciola spp. infection in different species of snails*

L. viridis showed the highest prevalence of larval *Fasciola* spp. infection followed by *L. auricularia* race *rufescens* and *L. auricularia sensu stricto* (Table 4.2.5). The Chi-square test (with Yates correction) indicated that there was a significant difference in the prevalence between *L. viridis* and *L. auricularia* race *rufescens* (χ^2_1 = 4.96, P = 0.026) but not between *L. viridis* and *L. auricularia sensu stricto* (χ^2_1 = 3.03, P = 0.081) or between *L. auricularia* race *rufescens* and *L. auriculatia sensu stricto* (χ^2_1 = 0.33, P = 0.695). None of the 1209 *L. luteola* examined in 13 months was found to be infected with *Fasciola* spp.

Table 4.2.5. Prevalence of *F. gigantica* infection in different species of *Lymnaea* snails.

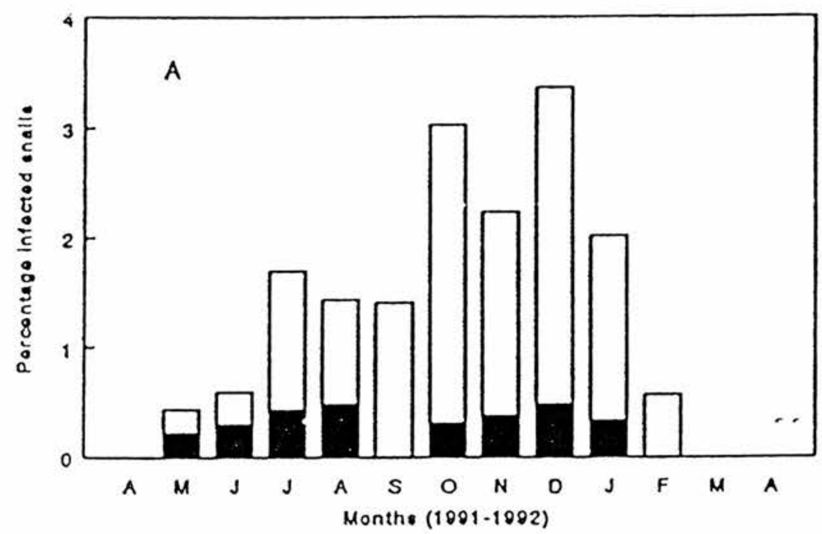
Species of snails	Number of snails examined	Number of snails positive	Percentage positive
L. auricularia race *rufescens*	5184	54	1.04
L. auricularia sensu stricto	691	5	0.72
L. viridis	1556	28	1.80
L. luteola	1209	0	0.00
Total	8640	87	1.01

Parasitism in snails with xiphidiocercariae and *Chaetogaster* spp. occurred more commonly in the hills (Appendix Table 4.2.9) than in the Terai (Appendix Table 4.2.10). Mixed infections with *F. gigantica* and *Chaetogaster* spp. were rarely seen while infection with *F. gigantica* and xiphidiocercariae was never recorded. Generally, snails smaller than 8 mm, were free from *Fasciola* spp. or xiphidiocercariae infections.

4.2.8 *Seasonal prevalence of F. gigantica infection in Lymnaea spp.*

In the hills, the prevalence of immature infection (redial stage) in the snails varied from 0.00% to 0.48% with the peaks in August and December; no immature infection was recorded in September and again from February to March (Appendix Table 4.2.9 and Figure 4.2.14). The Chi-square test showed that the monthly variation in the prevalence of immature infection was not significant (χ^2_{12} = 7.52, P = 0.822). The mature infections (redial+cercarial stages) were first detected in May (Appendix Table 4.2.9 and Figure 4.2.14). The prevalence of mature infection increased to reach the highest level of 2.88% in December. There was then a decline in the prevalence and no snail with the mature infection was found in March and April. There was an extremely significant monthly variation in the prevalence of mature infections in the snails (χ^2_{12} = 44.45, P = < 0.001).

In the Terai, the highest prevalence (0.67%) of immature infection was recorded in June (Appendix Table 4.2.10 and Figure 4.2.14). Immature infections were not found in the snails during August-November and again in January and February. Mature infections were recorded every month with a gradual rise in prevalence from April to July (Appendix Table 4.2.10 and Figure 4.2.14). The prevalence declined in August but rose again to the highest level of 1.81% in September. The monthly variations in the prevalence of neither immature nor mature infections were significant (χ^2_{12} = 9.50, P = 0.660 and χ^2_{12} = 11.50, P = 0.487 respectively).



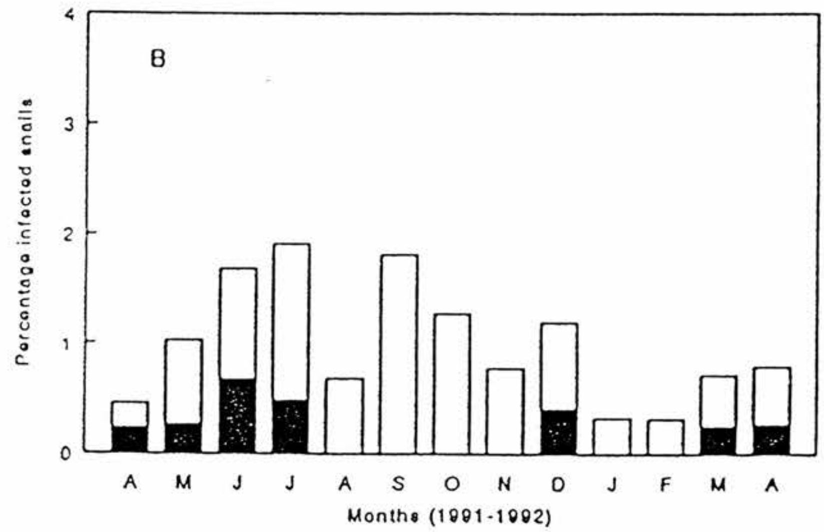


Figure 4.2.14. Seasonal prevalence of larval *Fasciola* spp. infection in *Lymnaea* snails in the hills (A) and the Terai (B); redia (■) and redia + cercaria (□).

4.3 Laboratory Based Experiments on the Ability of *L. auricularia* race *rufescens* and *L. viridis* to Survive in Drought Conditions

The snails produced many egg masses before becoming inactive within a few days after syphoning off the water from the trays. A few snails crawled out of the trays, but none of them buried themselves into the mud. The results of experiment 1 are presented in Table 4.3.1.

Table 4.3.1. Recovery of live snails after 15 and 30 days aestivation in dry mud in experiment 1.

Species of snails	Tray	Number of snails in the tray	Observations					
			Day 15			Day 30		
			Soil moisture (%)	Live snails (No.)	Survival (%)	Soil moisture (%)	Live snails (No.)	Survival (%)
L. auricularia race *rufescens*	C-1	38	ND	37	97.4	ND	31	81.6
	C-2	30	ND	28	93.3	ND	25	83.3
	D-1	33	48.6	11	33.3	-	-	-
	D-2	31	42.4	9	29.0	-	-	-
	D-3	30	ND	ND	ND	30.5	3	10.0
	D-4	35	ND	ND	ND	34.5	4	11.4
L. viridis	C-1	38	ND	38	100.0	ND	37	97.4
	C-2	35	ND	35	100.0	ND	33	94.3
	D-1	40	39.0	40	100.0	-	-	-
	D-2	32	45.0	32	100.0	-	-	-
	D-3	36	ND	ND	ND	30.8	34	94.4
	D-4	34	ND	ND	ND	32.0	31	91.2

Note: the tray numbers with initial C denotes control trays i.e. the trays which contained water and D denotes the trays from which water was syphoned off; ND = not determined; - = observations not continued after 15th days.

After day 15, the superficial layer of the mud became dry with the moisture content ranging from 39.0 to 48.6%, trays D-1 and D-2 were flooded with spring water. Three to four hours later motile snails were observed in each tray. While the survival rate was 100.0% for *L. viridis* in both the control and the dry trays, it varied from 29.0 to 33.3% in the dry and from 93.3 to 97.4% in the control trays for *L. auricularia* race *rufescens*. At day 30, the survival rate for *L. auricularia* race *rufescens* was significantly lower in the dry trays than in the controls (χ^2_1 = 24.63, P = < 0.001). However, the difference in the survival rates was not significant for *L. viridis* (χ^2_1 = < 1.0, P = 0.968).

In experiment 2, observations were made only at day 30 when the mud in the trays from which water was syphoned off had dried out completely and had shrunk, fissured and become hard. The results are presented in Table 4.3.2.

Table 4.3.2. Recovery of live snails after 30 days aestivation in dry mud in experiment 2.

Species & size of snails	Tray	Day 0		Day 30		
		Soil moisture (%)	Live snails (No.)	Soil moisture (%)	Live snails (No.)	Survival (%)
L. auricularia race *rufescens* (16-20 mm)	C-1	ND	38	ND	29	76.3
	C-2	ND	33	ND	27	81.8
	D-1	50.9	34	7.6	0	0.0
	D-2	44.3	32	5.8	0	0.0
	D-3	46.2	36	6.0	0	0.0
	D-4	48.0	35	6.5	0	0.0
L. auricularia race *rufescens* (4-5 mm)	C-1	ND	40	ND	35	87.5
	C-2	ND	36	ND	33	91.7
	D-1	46.8	34	6.4	13	38.2
	D-2	44.6	38	5.8	10	26.3
	D-3	47.4	36	6.3	12	33.3
	D-4	45.3	34	5.4	9	26.5
L. viridis (8-10 mm)	C-1	ND	35	ND	35	100.0
	C-2	ND	38	ND	33	92.1
	D-1	41.9	35	5.8	15	42.9
	D-2	45.4	37	5.7	16	43.2
	D-3	44.0	36	6.0	17	47.2
	D-4	48.3	39	7.9	19	48.7

Note: the tray numbers with initial C denotes control trays i.e. the trays which contained water and D denotes the trays from which water was syphoned off; ND = not determined.

At day 30, none of the mature *L. auricularia* race *rufescens* (16-20 mm) were alive in the dry mud but 76.3 to 81.8% of the controls survived. However, 26.3 to 38.2% of the young experimental *L. auricularia* race *rufescens* (4-5 mm) survived. In the dry mud, the mean survival rate of *L. viridis* (45.6%) was higher than that of the young *L. auricularia* race *rufescens* (31.0%), however, the Chi-square test (employing Yates correction) showed that the difference was not significant (χ^2_1 = 2.54, P = 0.111).

4.4 Farm/Flock Surveys Data

The raw data of the cross sectional and longitudinal surveys of farms and flocks are recorded in Appendix Tables 4.4.1-4.4.21.

4.4.1 *Prevalence of fasciolosis in cattle*

The overall prevalences as detected by faecal egg output in a total of 786 cattle sampled in the hills and 424 in the Terai were 44.8 and 51.4% respectively; the Chi-square test showed that the difference was not significant (χ^2_1 = 1.59, P = 0.2075). Details of the prevalences and faecal egg counts in cattle stratified by altitude, feeding system, age and sex, together with the results of statistical comparisons are shown in Tables 4.4.1 (hills) and 4.4.2 (Terai).

In the hills, the highest prevalence of fasciolosis in cattle was observed at the middle altitudes (56.6%) followed by the low (36.3%) and the high (29.0%) altitudes. While there were significant differences between these prevalences; differences in the median EPG values were not significant. When the data was analyzed according to the feeding systems, a lower prevalence was found in the stall fed animals than in the grazed ones; however, the difference was not significant. Taking into account the ages of the cattle sampled, it was found that the prevalence increased with age up to 5-6 years, remained at more or less the same levels for up to 9-10 years and then declined in the older animals. While differences in the prevalence among the different age groups were extremely significant, this was not the case with their median EPG values. Analysis showed that fasciolosis was significantly more frequent in males (50.4%) than females (40.0%) with a marginally significant difference in the EPG values.

In the Terai, the prevalence varied from 46.5% in the Tarahara Village Development Committee (VDC) area to 59.5% in the Letang VDC. The influence of feeding systems on the prevalence could not be ascertained because the number of stall-fed cattle was not large enough for the analysis. The age and sex ratio in prevalence followed a similar pattern to the hills. Although there were no significant variations in the area, age or sex-wise prevalence, the median EPG values varied significantly among animals from the different areas and the different age groups.

Table 4.4.1. Prevalence of fasciolosis in cattle in the hills (January-February 1991).

Variable	Group	Number of animals		Prevalence		Faecal egg counts (EPG)		
		Examined	+ ve	%	Statistic	Range	Median	Statistic
Altitude	High	221	64	29.0	χ^2 = 19.58	2-45	4.1	H = 4.89
	Mid	408	231	56.6	P = <0.0001	1-121	5.2	P = 0.0860
	Low	157	57	36.3		2-61	6.1	
Feeding system	Stall-fed	53	18	34.0	χ^2 = 0.84	1-48	5.2	W = 44468.5
	Grazed	733	334	45.6	P = 0.3587	1-121	6.5	P = 0.8934
Age (years)	< 1	24	3	12.5		4-7	6.1	
	1-2	158	53	33.5		1-69	5.2	
	3-4	143	71	49.7	χ^2 = 16.50	1-121	5.2	H = 7.59
	5-6	146	80	54.8	P = <0.0113	1-83	6.1	P = 0.2710
	7-8	147	72	49.0		1-112	4.6	
	9-10	114	60	52.6		1-91	2.6	
	11 & over	54	13	24.1		1-35	5.1	
Sex	Male	359	181	50.4	χ^2 = 2.99	1-83	6.1	W = 25600.5
	Female	427	171	40.0	P = 0.0836	1-121	4.1	P = 0.0516
Overall		786	352	44.8	NA	1-121	5.2	NA

Note: χ^2 = Chi-square statistic, H = Kruskal-Wallis statistic, W = Wilcoxon-Mann-Whitney statistic, P = P value (significance).

Table 4.4.2. Prevalence of fasciolosis in cattle in the Terai (March 1991).

Variable	Group	Number of animals		Prevalence		Faecal egg counts (EPG)		
		Examined	+ ve	%	Statistic	Range	Median	Statistic
Village	Tarahara	144	67	46.5		1-14	3.0	
	Letang	108	64	59.3	χ^2 = 1.57	1-129	7.0	H = 25.39
	Jhumka	95	45	47.4	P = 0.6666	2-95	4.0	P = <0.0001
	Bhantabari	77	42	54.5		1-52	5.0	
Age (years)	< 1	12	3	25.0		3-13	5.0	
	1-2	72	32	44.4		1-23	5.0	
	3-4	68	28	41.2	χ^2 = 6.18	1-30	4.0	H = 15.11
	5-6	80	51	63.8	P = 0.4038	1-91	5.0	P = 0.0201
	7-8	76	48	63.2		1-129	7.0	
	9-10	55	30	54.5		1-18	3.0	
	11 & over	61	26	42.6		1-52	4.0	
Sex	Male	236	126	53.4	χ^2 = 0.19	1-129	4.0	W = 13272.0
	Female	188	92	48.9	P = 0.6649	1-96	5.0	P = 0.2501
Overall		424	218	51.4	NA	1-129	5.0	NA

Note: χ^2 = Chi-square statistic, H = Kruskal-Wallis statistic, W = Wilcoxon-Mann-Whitney statistic, P = P value (significance).

4.4.2 *Seasonal incidence and prevalence of fasciolosis in cattle*

Monthly incidence of patent fasciolosis in cattle was monitored using 59 tracer calves in the hills and 60 in the Terai. During certain months, the number of animals at risk was not large enough to determine the altitude/area-wise incidence (Appendix Tables 4.4.18 and 4.4.19), therefore only the overall incidence in the hills and the Terai are shown in Figures 4.4.1 and 4.4.2 respectively. Seasonal changes in the prevalence of chronic fasciolosis which were assessed using 60 cattle in the hills (low and mid altitudes) and 158 cattle in the Terai are also shown in Figures 4.4.1 and 4.4.2.

In the hills, the incidence varied from 0.0% in November to 35.1% in August. Although, seasonal peaks were seen in May (22.4%), August (35.1%) and February (31.6%), the Chi-square test indicated that the monthly variation was not significant (χ^2_{12} = 19.66, P = 0.0739). In general, the overall trend of the seasonal prevalence was similar to that of the seasonal incidence. However, some differences were observed between the trends of the seasonal prevalence in the low and the mid altitude areas. In the low altitude area, there were erratic rises and falls in the prevalence during May-September with marked peaks in May and August. Thereafter, the prevalence rose in October reaching a peak in December and then gradually declined until March. In this area, the highest prevalence (56.5%) was recorded in December while the lowest (9.5%) was in June. In the mid altitude area, however, there was no rise in the prevalence in May, but the peaks were seen in September (35.7%) and January (41.7%) while the lowest prevalence (10.3%) was recorded in October. The Chi-square test showed that monthly variation in the prevalence was significant neither in the low nor in the mid altitude areas (χ^2_{12} = 14.64, P = 0.2616 and χ^2_{12} = 6.96, P = 0.8602 respectively). The mean faecal egg counts ranged from 1.6 EPG in September to 12.7 EPG in February in the low altitude area and from 1.5 EPG in May to 7.9 EPG in February in the mid altitude area. Except for a high mean EPG value in June in the low altitude area, the counts were generally higher during December-March in both areas.

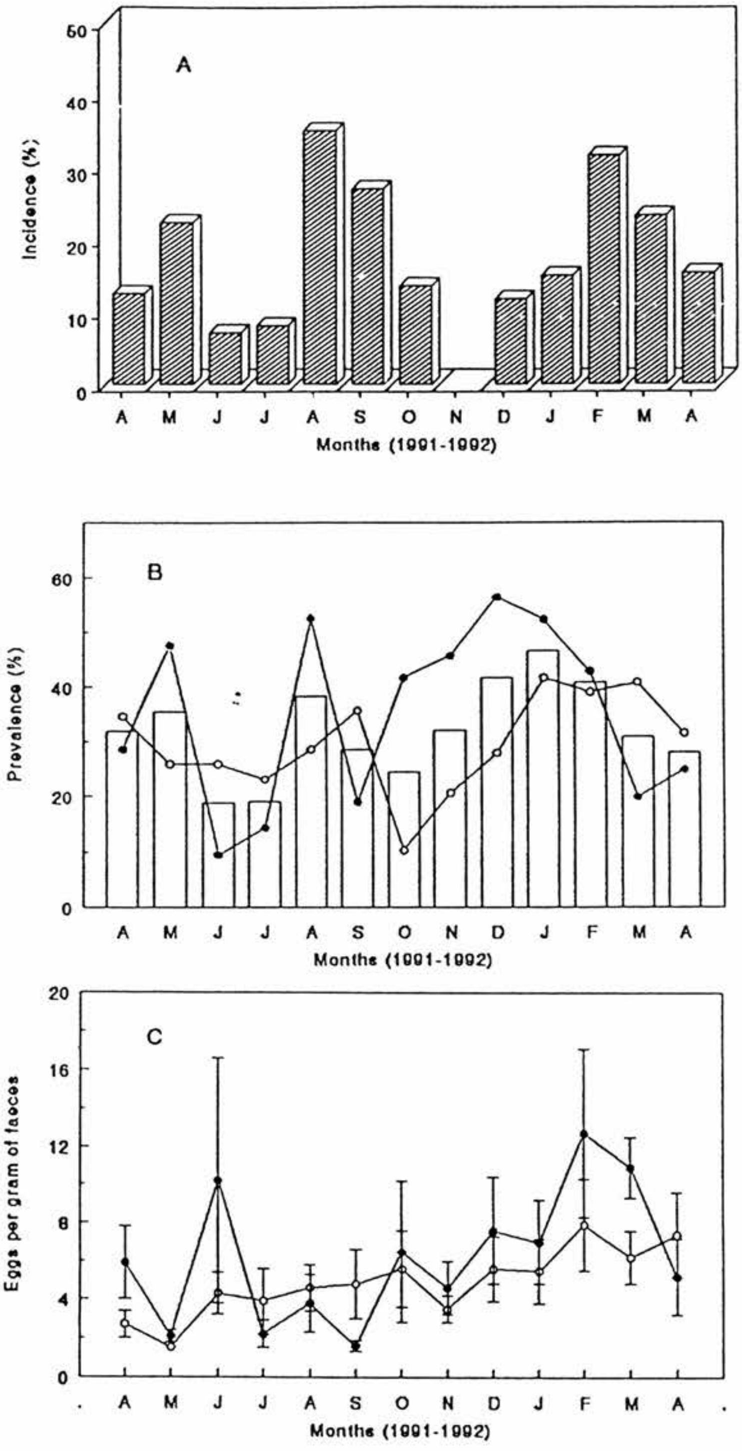


Figure 4.4.1. Seasonal incidence of fasciolosis in tracer zebu calves in the hills (A), seasonal prevalence (B) and fluctuation in the mean ± SE faecal egg counts (C) in cattle in the low (●) and the mid (○) altitude hills. Bars in figure (B) show the overall seasonal prevalence in cattle in the hills.

In the Terai, the incidence varied from 0.0% in November, December and April (1992) to 34.7% in July. Higher incidences were recorded in July-August, October and again during January-March. The Chi-square test showed that the monthly variation was significant (χ^2_{12} = 33.80, P = 0.0007). There were low overall prevalences in April-June which rose in July, gradually fell until December, rose again in January-February and again fell in March-April. Similar trends in the prevalence with significant monthly variations were observed in all the three areas, namely Tarahara (χ^2_{12} = 25.10, P= 0.0143), Letang (χ^2_{12} = 33.71, P = 0.0008) and Jhumka/Bhantabari (χ^2_{12} = 26.87, P = 0.0081). There was no uniformity in the monthly mean EPG values in these areas, while, the highest count was recorded in July in Tarahara (13.4) this occurred in November in Letang (12.7) and in December in Jhumka/Bhantabari (11.8).

4.4.3 *Prevalence of fasciolosis in buffaloes*

There was a significantly higher prevalence of fasciolosis in buffaloes in the hills (57.9%) than the Terai (41.3%) (χ^2_1 = 5.52, P = 0.0188). In the hills, the prevalence varied significantly at the three altitude ranges, the highest was recorded in the mid altitude area and the lowest in the high altitude area (Table 4.4.3). Although, the median EPG values were higher in the mid altitude area, the differences were not significant. Unlike cattle, the prevalence as well as the median EPG value were higher in stall-fed buffaloes than grazed ones. While there was a significant difference in prevalence, the median EPG values were not significantly different. There was an increase in the prevalence in buffaloes with advancing age, however there were no significant differences either in the prevalence or the median EPG among the different age groups. Although, there was a higher prevalence of 61.3% in female buffaloes compared to 35.6% in males, this difference was not significant.

Details of the prevalence in buffaloes in the Terai are presented in Table 4.4.4. The highest prevalence was recorded in Tarahara followed by Letang and Jhumka/Bhantabari (only a few buffaloes were sampled in Jhumka, therefore the data were pooled). While the prevalence between these VDC area was significantly different, the median EPG values were not significantly different. As in the hills,

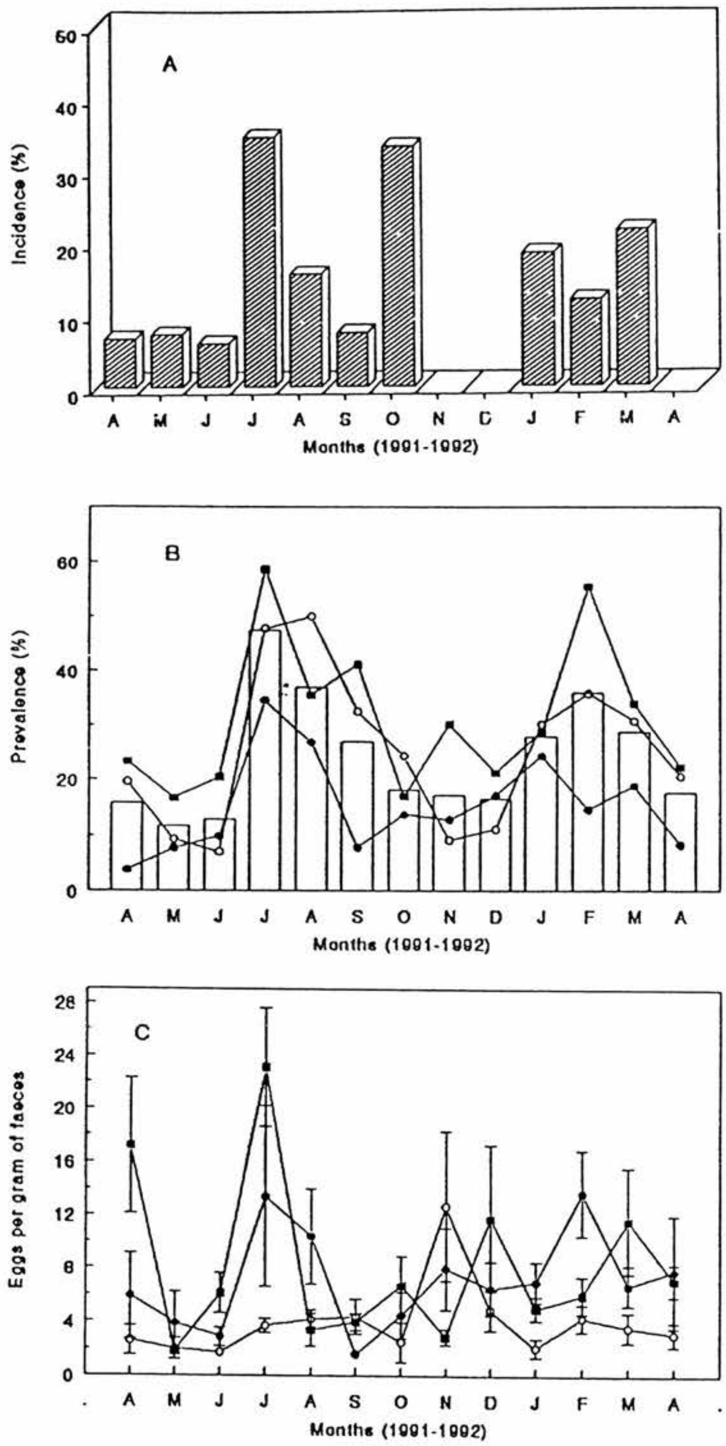


Figure 4.4.2. Seasonal incidence of fasciolosis in tracer zebu calves in the Terai (A), seasonal prevalence (B) and fluctuation in the mean ± SE faecal egg counts (C) in cattle in Tarahara (●), Letang (○) and Jhumka/Bhantabari (■) VDCs. Bars in figure (B) show the overall seasonal prevalence in cattle in the Terai.

the prevalence increased with age, however there were no significant differences either in the prevalence or in the median EPG values among the different age groups. Again, female buffaloes had a higher prevalence of fasciolosis than males, although not significantly different.

Table 4.4.3. Prevalence of fasciolosis in buffaloes in the hills (January-February 1991).

Variable	Group	Number of animals		Prevalence		Faecal egg counts (EPG)		
		Examined	+ ve	%	Statistic	Range	Median	Statistic
Altitude	High	141	59	41.8	χ^2 = 7.14	1-115	7.8	H = 0.57
	Mid	168	118	70.2	P = 0.0281	1-254	13.7	P = 0.7510
	Low	28	18	64.3		2-85	7.8	
Feeding system	Stall-fed	160	111	69.4	χ^2 = 4.04	1-254	11.7	W = 4231.0
	Grazed	177	84	47.5	P = 0.0444	1-163	6.5	P = 0.2003
Age (years)	< 1	17	3	17.6		1-5	3.3	
	1-2	51	22	43.1		1-254	9.1	
	3-4	76	44	57.9	χ^2 = 7.06	1-163	10.4	H = 4.22
	5-6	45	25	55.6	P = 0.3155	1-90	9.1	P = 0.6472
	7-8	59	40	67.8		1-104	4.6	
	9-10	52	35	67.3		1-73	9.1	
	11 & over	37	26	70.3		1-217	6.5	
Sex	Male	45	16	35.6	χ^2 = 2.74	1-115	9.1	W = 716.5
	Female	292	179	61.3	P = 0.0980	1-254	7.8	P = 0.8020
Overall		337	195	57.9	NA	1-254	8.5	NA

Note: χ^2 = Chi-square statistic, H = Kruskal-Wallis statistic, W = Wilcoxon-Mann-Whitney statistic, P = P value (significance).

Table 4.4.4. Prevalence of fasciolosis in buffaloes in the Terai (March 1991).

Variable	Group	Number of animals		Prevalence		Faecal egg counts (EPG)		
		Examined	+ ve	%	Statistic	Range	Median	Statistic
Village	Tarahara	138	67	48.6	χ^2 = 7.72	1-151	6.1	H = 1.72
	Letang	94	43	45.7	P = 0.0210	2-106	4.6	P = 0.4231
	Bhan Jhum	66	13	19.7		1-18	3.0	
Age (years)	< 1	38	7	18.4		1-11	3.9	
	1-2	39	11	28.2		1-53	3.5	
	3-4	32	10	31.3	χ^2 = 9.40	1-107	5.4	H = 4.94
	5-6	34	13	38.2	P = 0.1522	1-47	3.9	P = 0.5513
	7-8	54	27	50.0		1-91	4.6	
	9-10	47	25	53.2		1-55	6.1	
	11 & over	54	30	55.6		1-151	3.0	
Sex	Male	69	24	34.8	χ^2 = 0.48	1-107	3.5	W = 1332.5
	Female	229	99	43.2	P = 0.4902	1-151	4.6	P = 0.3199
Overall		298	123	41.3	NA	1-151	4.3	NA

Note: χ^2 = Chi-square statistic, H = Kruskal-Wallis statistic, W = Wilcoxon-Mann-Whitney statistic, P = P value (significance); Bhan Jhum = Bhantabari and Jhumka VDC.

4.4.4 *Seasonal incidence and prevalence of fasciolosis in buffaloes*

In the hills, except for June and August, new patent infections in the tracer buffalo calves with *Fasciola* spp. occurred every month (Figure 4.4.3). The incidence was high in October (43.8%), November (55.6%), February (30.0%) and April (40.0%). The Chi-square test showed that there was a significant monthly variation in the incidence (χ^2_{12} = 22.73, P = 0.0301). The overall prevalence varied from 29.8% in October to 61% in March (Figure 4.4.3). In the low altitude area there were three peaks, in August (46.2%), November (73.1%) and March (72.7%). However, the monthly variation was not significant (χ^2_{12} = 7.29, P = 0.8373). In the mid altitude area, the monthly prevalences were lower than the low altitude area but there was a similar trend until October. In November, unlike in the low altitude area, there was a further decline but the prevalence rose gradually thereafter until April. In this area also, the monthly variation in the prevalence was not significant (χ^2_{12} = 3.41, P = 0.9919). The mean EPG varied from 2.6 in September to 14.1 in February in the low altitude area and from 2.8 in July to 15.6 in December in the mid altitude area (Figure 4.4.3).

In the Terai, incidence of patent fasciolosis occurred throughout the year with a peak of 41.7% in February and a minimum of 8.7% in November (Figure 4.4.4). The Chi-square test showed that variation in the monthly incidence was not significant (χ^2_{12} = 10.37, P= 0.5837). The overall prevalence varied from 40.4% in March to 69.3% in August (Figure 4.4.4). In all the three areas, generally there were rises during the mid to late monsoon (July-September) and again during the mid dry season (December-February). However, the monthly variations were not significant (χ^2_{12} = 7.27, P = 0.8396 for Tarahara; χ^2_{12} = 9.31, P = 0.6763 for Letang; χ^2_{12} = 10.57, P = 0.5659 for Jhumka/Bhantabari). There was no uniformity in the mean EPG values in the three sampling areas; while the highest EPG was found in June in Letang (24.0), this was in November in Tarahara (22.8) and December in Jhumka/Bhantabari (19.4) (Figure 4.4.4).

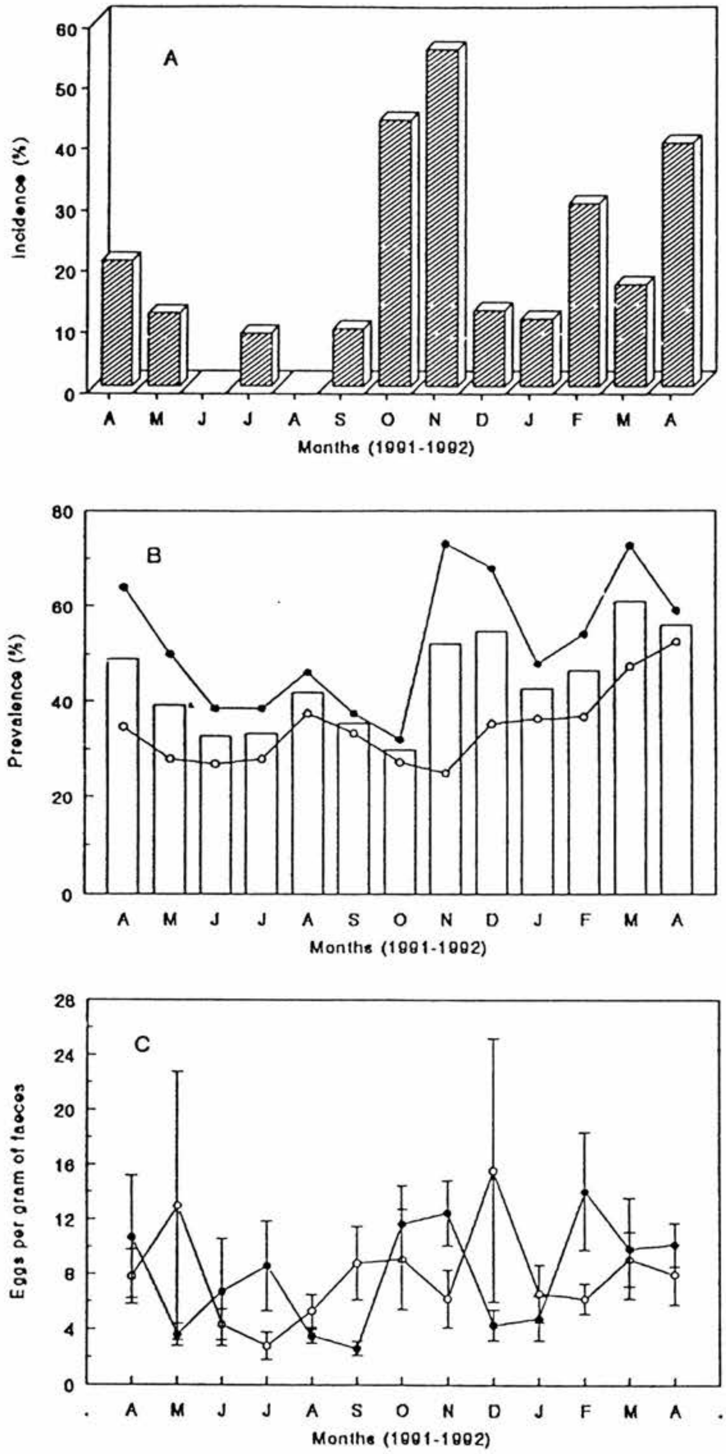


Figure 4.4.3. Seasonal incidence of fasciolosis in tracer buffalo calves in the hills (A), seasonal prevalence (B) and fluctuation in the mean ± SE faecal egg counts (C) in buffalo in the low (●) and the mid (O) altitude hills. Bars in figure (B) show the overall seasonal prevalence in buffalo in the hills.

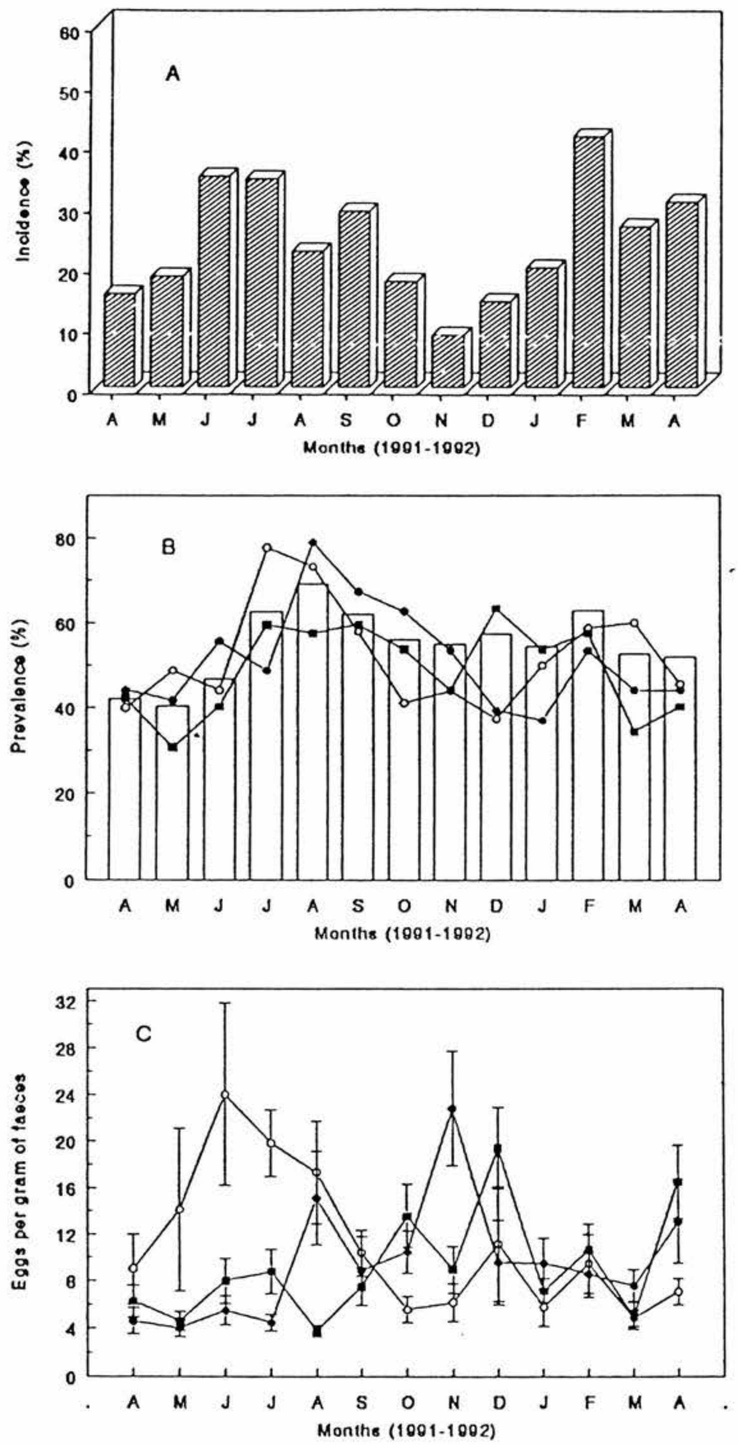


Figure 4.4.4. Seasonal incidence of fasciolosis in tracer buffalo calves in the Terai (A), seasonal prevalence (B) and fluctuation in the mean ± SE faecal egg counts (C) in buffaloes at Tarahara (●), Letang (○) and Jhumka/Bhantabari (■) VDCs. Bars in figure (B) show the overall seasonal prevalence in buffaloes in the Terai.

4.4.5 *Prevalence of fasciolosis in goats*

The area, age and sex distributions of fasciolosis in goats in the hills and the Terai are presented in Tables 4.4.5 and 4.4.6 respectively.

There was a significantly higher prevalence of fasciolosis in goats in the hills (22.4%) than the Terai (13.3%) (χ^2_1 = 4.77, P = 0.0289). In the hills, the highest prevalence was found in the mid altitude and the lowest in the high altitude area. There were significant differences in the prevalence between the three areas; however, the median EPG values were not significantly different. The age distribution showed that the prevalence increased significantly with advancing age; however, differences in the EPG values were not significant. Female goats were significantly more frequently affected than males, although, with an insignificant difference in the median EPG.

In the Terai, the highest prevalence was found in Bhantabari/Jhumka, followed by Tarahara and Letang. However, differences in the prevalence as well as the median EPG values between these areas were not significant. The trends in age and sex distributions were similar to those in the hills; however, neither the prevalence nor the EPG values were significantly different.

Table 4.4.5. Prevalence of fasciolosis in goats in the hills (January-February 1991).

Variable	Group	Number of animals		Prevalence		Faecal egg counts (EPG)		
		Examined	+ ve	%	Statistic	Range	Median	Statistic
Altitude	High	71	4	5.6	χ^2 = 14.27	5-29	7.8	H = 1.14
	Mid	315	90	28.6	P = 0.0281	1-124	5.2	P = 0.4952
	Low	127	21	16.5		3-29	5.2	
Age (years)	< 1	24	1	4.2		NA	5.2	
	1-2	201	29	14.4	χ^2 = 16.18	1-105	5.2	H = 2.39
	3-4	179	46	25.7	P = 0.0028	1-72	5.2	P = 0.4962
	5-6	74	24	32.4		1-124	6.5	
	7 & over	35	15	42.9		1-52	9.8	
Sex	Male	140	19	13.6	χ^2 = 5.21	1-29	5.2	W = 563.5
	Female	373	96	25.7	P = 0.0225	1-124	6.5	P = 0.1744
Overall		513	115	22.4	NA	1-124	6.5	NA

Note: χ^2 = Chi-square statistic, H = Kruskal-Wallis statistic, W = Wilcoxon-Mann-Whitney statistic, P = P value (significance).

Table 4.4.6. Prevalence of fasciolosis in goats in the Terai (March 1991).

Variable	Group	Number of animals		Prevalence		Faecal egg counts (EPG)		
		Examined	+ ve	%	Statistic	Range	Median	Statistic
Village	Tarahara	37	5	13.5	$\chi^2 = 2.16$	1-12	2.0	H = 0.48
	Letang	58	5	10.3	P = 0.3389	1-5	2.6	P = 0.7877
	Bhan/Jhum	33	7	21.2		1-26	2.6	
Age (years)	< 1	13	1	7.7		1-4	2.6	
	1-2	64	8	12.5	$\chi^2 = 1.77$*	1-5	2.4	H = 3.39
	3-4	30	3	10.0	P = 0.4130	1-12	3.9	P = 0.495
	5-6	15	3	20.0		1-8	2.6	
	7 & over	6	2	33.3		1-26	5.2	
Sex	Male	42	4	9.5	$\chi^2 = 0.25$	1-5	2.3	W = 16.5
	Female	86	13	15.1	P = 0.6203	1-26	4.2	p = 0.6728
Overall		128	17	13.3	NA	1-26	3.3	NA

Note: χ^2 = Chi-square statistic, H = Kruskal-Wallis statistic, W = Wilcoxon-Mann-Whitney statistic, P = P value (significance); * = d.f. only 2 because both extremes data were combined to the nearest group in order to make the analysis valid; Bhan/Jhum = Bhantabari and Jhumka VDC.

4.4.6 *Seasonal prevalence of fasciolosis in goats*

In the hills, the prevalences in both the low and mid altitude areas were higher in August and again from January to April than other months, with the peaks in August (24.0 and 21.4% respectively) and March 27.8 and 30.0% respectively) (Figure 4.4.5). However, variations in the monthly prevalence were not significant (χ^2_{12} = 3.16, P = 0.9943 for low altitude and χ^2_{12} = 3.41, P = 0.9919 for mid altitude).

In the Terai, the seasonal prevalence of fasciolosis in goats was studied only in Letang VDC area. After reaching a peak in June, the prevalence declined in July and fluctuated at about the same level until January (Figure 4.4.6). In February, the level increased reaching a second peak in March and again decreased in April. However, the Chi-square test showed that the monthly variation was not significant (χ^2_{12} = 12.53, P = 0.4042). The mean EPG values ranged from 2.1 to 13.2 with the peaks in August-September and March.

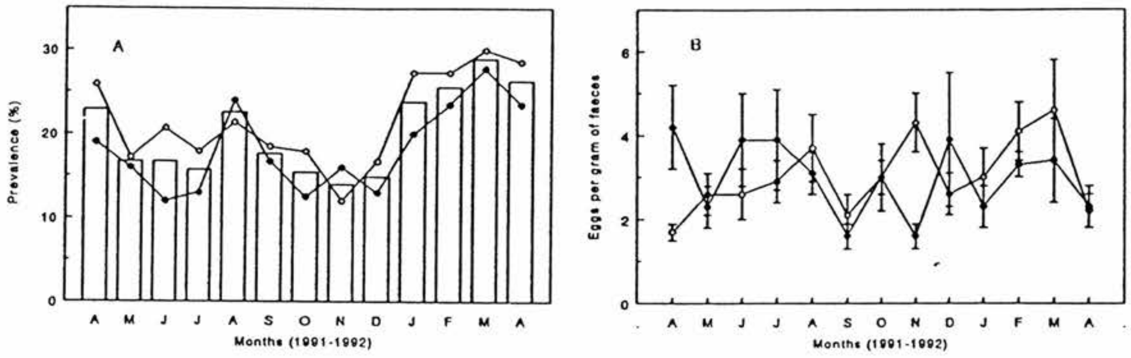


Figure 4.4.5. Seasonal prevalence (A) and fluctuation in the mean ± SE faecal egg counts (B) in goats in the low (●) and mid (○) altitude hills. Bars in figure (A) show the overall seasonal prevalence in goats in the hills.

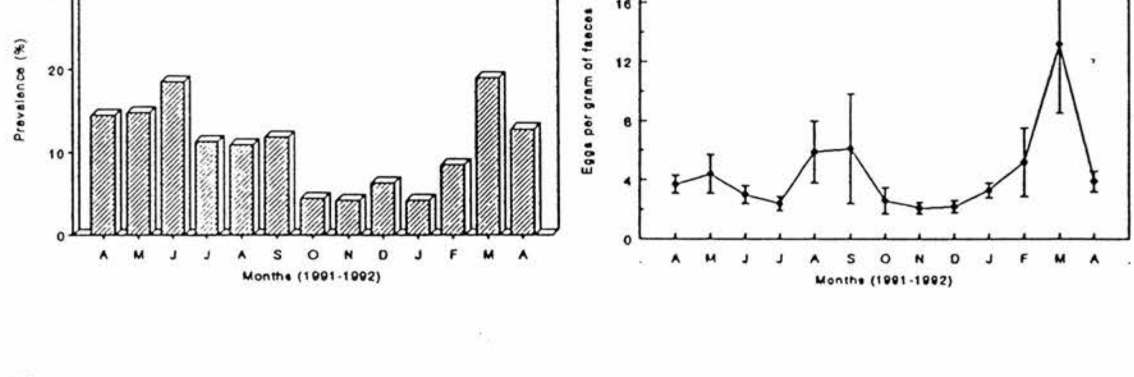


Figure 4.4.6. Seasonal prevalence (A) and fluctuation in the mean ± SE faecal egg counts (B) in goats in Letang VDC in the Terai.

4.4.3 *Prevalence of fasciolosis in sheep*

Prevalence of fasciolosis in sheep was studied only in the hills because sheep farming is not practised within the study areas in the Terai. The production system, age and sex distribution of fasciolosis in sheep in the hills are given in Table 4.4.7.

Table 4.4.7. Prevalence of fasciolosis in sheep in the hills (February 1991).

Variable	Group	Number of animals		Prevalence		Faecal egg counts (EPG)		
		Examined	+ ve	%	Statistic	Range	Median	Statistic
Production system	Migratory	234	35	14.9	χ^2 = 5.74	1-39	5.2	W = 445.0
	Sedentary	41	15	36.5	P = 0.0166	5-68	15.2	P = 0.0037
Age (years)	< 1	28	2	7.1		1-4	2.6	
	1-2	58	7	12.1	χ^2 = 4.99	5-47	7.8	H = 6.04
	3-4	66	11	16.7	P = 0.2882	3-47	7.8	P = 0.1972
	5-6	42	10	23.8		3-68	10.4	
	7 & over	81	20	24.7		3-39	5.2	
Sex	Male	40	5	12.5	χ^2 = 0.40	3-20	9.1	W = 80.5
	Female	235	45	19.1	P = 0.5264	1-47	7.8	P = 1.0000
Overall		275	50	18.2	NA	1-68	7.8	NA

Note: χ^2 = Chi-square statistic, H = Kruskal-Wallis statistic, W = Wilcoxon-Mann-Whitney statistic, P = P value (significance).

The prevalence as well as the median EPG values were significantly higher in sedentary sheep than migratory ones. There was an increase in the prevalence with advancing age; however, neither the prevalence nor the median EPG values between the different age groups were significantly different. Fasciolosis was more frequently encountered in female sheep than males; however, there were no significant differences in the prevalence and the Median EPG values between the two groups.

4.5 Slaughter Place Survey Data

Raw data for the slaughter place survey are recorded in Appendix Table 4.5.1.

4.5.1 *Prevalence of fasciolosis in slaughtered buffaloes and goats*

Of the total of 408 buffaloes and 39 goats slaughtered at Hile and Budhabare/PAC between January 1991 and July 1992, the overall rate of infection with *Fasciola* spp. was 85.0% (347) and 35.9% (14) respectively. In the Terai, a total of 150 buffalo livers were examined at the Letang *hat-bazaar* between August 1991 and May 1992 of which 66.7% (100) were infected with *Fasciola* spp. While storing the data in computer, the information on area of origin, age and sex of those animals whose livers were not brought to the PAC laboratory were omitted accidently. Therefore, influence of these variables on the prevalence could not be elucidated.

4.5.2 *Fluke burdens in buffaloes, goats and sheep*

The overall burdens of *Fasciola* spp. were 203.2 ± 17.9 and 23.7 ± 3.3 per infected buffalo and goat liver respectively. Only three sheep livers were available for examination, in which 35, 60 and 65 flukes were found.

Table 4.5.1. Burdens of *Fasciola* spp. in infected buffaloes.

Variable	Group	Number of livers examined	Fluke burdens				Statistic
			Range	Mean	S.E.	Median	
Area	High hills	6	9-550	247.0	88.1	185.5	
	Mid hills	65	1-2071	218.5	38.4	152.0	H = 7.53
	Low hills	62	2-771	167.1	19.1	124.0	P = 0.0569
	Terai	30	22-500	235.6	23.0	228.0	
Age (years)	1-2	26	1-597	77.1	24.1	40.0	
	3-4	18	13-550	144.3	28.5	144.0	
	5-6	16	30-771	260.1	53.2	197.5	H = 32.73
	7-8	30	6-760	185.6	30.1	162.5	P = <0.0001
	9-10	39	48-787	242.7	23.3	215.0	
	11 & over	34	3-2071	274.1	63.8	207.5	
Sex	Male	63	1-597	138.0	16.9	106.0	U = 2120.0
	Female	100	3-2071	244.1	26.3	195.0	P = 0.0005
Overall		163	1-2071	203.2	17.9	160.0	NA

Note: H = Kruskal-Wallis statistic, U = Mann-Whitney U statistic, P = P value (significance).

There were no significant differences in the area distribution of fluke burdens in buffaloes (Table 4.5.1). While considering the age distribution, heavier fluke burdens appeared to occur in older animals; there were significant differences between the age groups. Also, there was a pronounced sex bias in the fluke burdens with significantly higher numbers of flukes in female buffaloes than males.

4.5.3 *Seasonal variation in the occurrence and number of F. gigantica in the livers of infected buffaloes*

There was no marked seasonal variation in the rate of infection in buffaloes in the hills, however, the rates varied from 33.0% in December to 85.7% in September in the Terai (Table 4.5.2). The seasonal infection rates in goats could not be determined because of scanty and irregular availability of livers for examination.

Table 4.5.2. Seasonal prevalence of fasciolosis in slaughtered buffaloes (1991-1992).

Month	Hills (Hile and Budhabare bazaar)			Terai (Letang bazaar)		
	No. examined	No. positive	% positive	No. examined	No. positive	% positive
Jan 91	17	14	82.4	-	-	-
Feb 91	18	14	77.8	-	-	-
Mar 91	10	9	90.0	-	-	-
Apr 91	8	6	75.0	-	-	-
May 91	16	14	87.5	-	-	-
Jun 91	11	11	100.0	-	-	-
Jul 91	9	7	77.8	-	-	-
Aug 91	17	13	76.5	20	8	40.0
Sep 91	22	22	100.0	42	36	85.7
Oct 91	9	8	88.9	6	4	66.7
Nov 91	12	12	100.0	12	9	75.0
Dec 91	20	18	90.0	3	1	33.3
Jan 92	18	16	88.9	20	16	80.0
Feb 92	24	19	79.2	12	9	75.0
Mar 92	43	40	93.0	10	4	40.0
Apr 92	68	49	72.1	25	13	52.0
May 92	34	33	97.1	-	-	-
Jun 92	29	25	86.2	-	-	-
Jul 92	23	17	73.9	-	-	-
Total	408	347	85.1	150	100	66.7

Note: in the Terai, the survey was carried out only during August 1991 - April 1992.

Seasonal variations in the occurrence and number of immature *F. gigantica* (25 mm or shorter in length were considered as immature) in the livers of infected buffaloes in the hills (not determined in the Terai) are shown in Table 4.5.3. Except for March and September 1991, immature *F. gigantica* were found throughout the 19 months study period, with the highest infection rate of 66.7% in June and July 1991. The mean immature fluke burdens ranged from 0.3 per liver in August to 177.0 per liver in January 1992. The highest number of 2000 immature flukes in a liver was recorded in January 1992. The buffalo had been stall fed on grass cut and carried from ricefields for the 4 months; after 2-3 weeks of serious illness, it was sold to a butcher.

Table 4.5.3. Seasonal variations in immature, mature and overall *Fasciola* spp. burdens in the livers of infected buffaloes.

Month	Number of liver examined	Immature flukes				Mature fluke		Overall	
		Occurrence		Burdens					
		No. +ve	% +ve	Mean	S.E.	Mean burdens	S.E.	Mean burdens	S.E.
Jan 91	7	3	42.9	31.1	15.9	254.0	70.8	287.0	85.1
Feb 91	6	1	16.7	1.7	1.5	174.1	74.7	175.8	74.2
Mar 91	4	0	0.0	-	-	232.7	61.2	232.7	61.2
Apr 91	4	1	25.0	0.8	0.6	160.5	47.9	161.2	47.4
May 91	5	1	20.0	0.8	0.7	316.0	104.8	316.8	104.6
Jun 91	3	2	66.7	0.7	0.5	140.0	41.9	140.6	41.5
Jul 91	3	2	66.7	110.3	69.4	564.3	186.6	674.6	202.0
Aug 91	9	1	11.1	0.3	0.2	179.1	44.9	179.4	44.8
Sep 91	14	0	0.0	-	-	223.9	57.6	223.9	57.6
Oct 91	6	1	16.7	0.5	0.5	90.7	29.8	91.2	29.6
Nov 91	7	1	14.3	2.4	2.2	232.4	58.1	234.8	57.1
Dec 91	8	3	37.5	1.3	0.6	147.6	46.2	148.8	46.1
Jan 92	12	4	33.3	177.0	158.8	149.0	32.3	326.0	155.8
Feb 92	11	2	18.2	1.2	0.8	220.2	32.8	221.4	33.1
Mar 92	10	3	30.0	13.3	8.5	204.8	35.6	218.1	39.9
Apr 92	17	6	35.3	28.5	25.2	119.0	25.4	147.5	37.2
May 92	18	3	16.7	2.3	1.7	173.1	28.3	175.4	28.0
Jun 92	11	4	36.4	1.0	0.5	119.9	37.4	120.9	37.7
Jul 92	8	2	25.0	3.0	2.4	96.9	36.0	99.9	36.4

There was no identifiable seasonal pattern of the mean mature and overall fluke burdens, although these were generally high throughout the year. Also, there was no consistency in the results obtained in the different years. For example, while the highest mean burden of 564.3 flukes was found in July 1991, the lowest mean burden of 96.9 flukes was recorded in the same month during the seven months study period in 1992.

4.5.4 *Species of flukes*

There were great morphological differences among the 4200 *Fasciola* spp. specimens which were collected from the livers of 128 infected animals (111 buffalo, 14 goats and 3 sheep). The specimens were, however, classified morphometrically as category A, B and C. In category A, the length and width of mature flukes ranged from 22.9 to 35.5 and 9.8 mm to 13.5 mm respectively with the length:width ratio ranging between 2.2-2.4:1. The adult flukes of category B were 28.5 to 39.8 mm in length and 9.2 to 11.3 in width with the ratio ranging between 3.1-3.6:1. The adult flukes of category C were the longest of all the categories, the length and width ranging from 28.8 to 63.6 mm and 7.2 to 11.1 mm respectively. The length:width ratio of these flukes ranged between 4.1-7.4:1. All these measurements were carried out on unfixed specimens.

Eggs were obtained from the unfixed fresh flukes by cutting a part of the body immediately posterior to the ventral sucker, where the uterus contains mature eggs. The results of egg measurements carried out on 500 eggs from the each category are presented in Table 4.5.4.

Table 4.5.4. Sizes of the eggs of the three categories of *Fasciola* spp.

Category	Length in μm			Width in μm		
	Range	Mean	S.E.	Range	Mean	S.E.
A	107.1-166.6	139.9	1.4	59.5-106.0	82.9	1.7
B	121.2-175.7	153.1	1.1	71.4-136.4	93.3	1.4
C	139.4-224.2	170.5	1.4	78.8-145.4	115.4	1.5

Considering the shape and the morphometric parameters described above, category A was identified as *F. hepatica* and C as *F. gigantica*, while category B appeared to be intermediate between these two species (Figure 4.5.1). Fifteen fixed specimens from the each category were sent to Dr Arlene Jones, International Institute of Parasitology, St Albans, England. She confirmed the identification of category A and C, however, she considered that category B is closest to *F. gigantica*, although the egg size is rather low for this species.

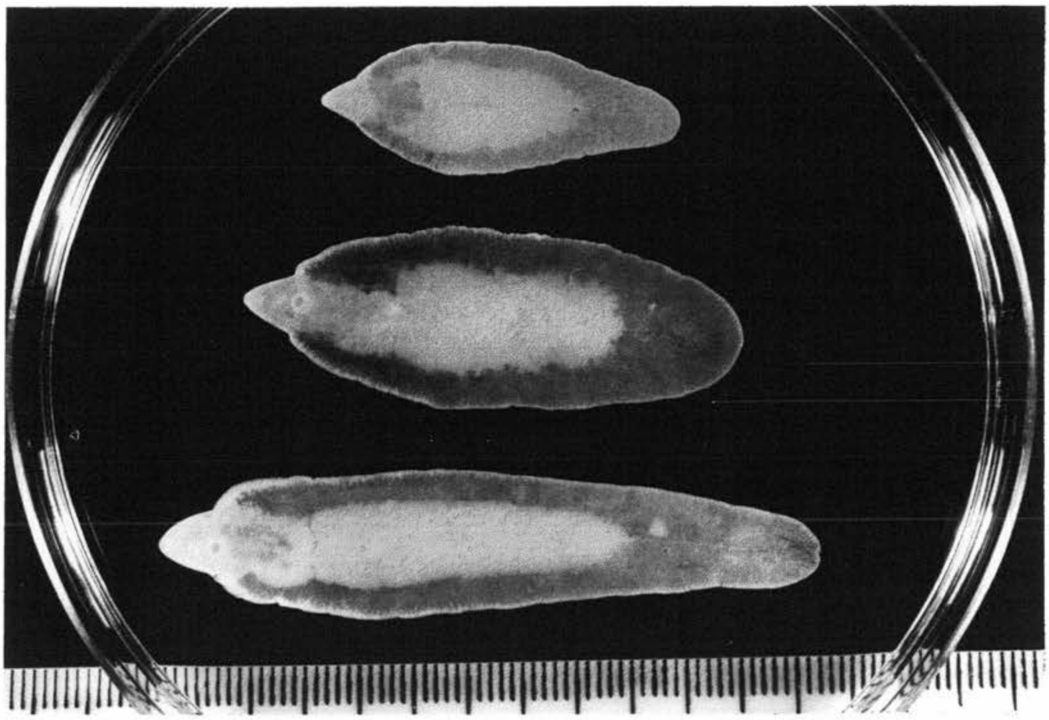


Figure 4.5.1. Unstained specimens of category A (*F. hepatica*)(top), category B (intermediate form)(middle) and category C (*F. gigantica*)(bottom).

Distributions of *Fasciola* spp. in the livers of infected buffaloes, goats and sheep are shown in Table 4.5.5. While single infection of *F. gigantica* was found in 89.8% of infected livers, either single or mixed infection of *F. hepatica* was found only in 1.6% and intermediate form in 8.6%. A mixed infection of *F. gigantica* and the intermediate form was more common than that of *F. gigantica* and *F. hepatica*. The intermediate forms were always found in mixed infections with *F. gigantica* only.

Table 4.5.5. Distribution of *Fasciola* spp. in the livers of infected buffalo, goat and sheep.

Host	Number of livers examined	*F. gigantica* only (No. of livers)	*F. hepatica only* (No. of livers)	Mixed *F. gigantica* and *F. hepatica* (No. of livers)	Mixed *F. gigantica* and Intermediate form (No. of livers)
Buffalo	111	102	0	0	9
Goats	14	11	0	1	2
Sheep	3	2	1	0	0
Total	128	115 (89.8%)	1 (0.8%)	1 (0.8%)	11 (8.6%)

While the vertical distribution of *F. hepatica* was confined to the mid and high altitude hills, *F. gigantica* was found widely distributed from the Terai to the high altitude hills. *Fasciola hepatica* exclusively was found in only one sheep which was purchased by PAC from the Pansayakhola Livestock Farm, located in the high hills of mid western Nepal. The intermediate form was recorded only in the mid altitude hills.

CHAPTER FIVE

DISCUSSION

5.1 Definitive Hosts

The present study clearly showed that fasciolosis caused by *F. gigantica* is very prevalent in ruminants in eastern Nepal. Although, infection with *F. hepatica* and an intermediate form were also recorded, these appeared to be not important. While *F. gigantica* was found to be widespread from the Terai to the high hills, *F. hepatica* and the intermediate form were found only in the middle and high hills. Furthermore, either in single or mixed infections, *F. hepatica* and the intermediate forms were found only in 1.6 and 8.6% of infected animals respectively. These findings are not in agreement with Lohani and Jaeckle (1981/82) who reported that *F. hepatica* and the intermediate form occurred in more than 60% of infected buffalo and goat livers at the high, middle and low altitude hills of western Nepal.

The overall prevalences as detected by faecal egg output in cattle (44.8% in the hills and 51.4% in the Terai), buffaloes (57.9% in the hills and 41.3% in the Terai), goats (22.4% in the hills and 13.3% in the Terai) and sheep (18.2% in the hills) are very similar to those recorded previously from the eastern hills of Nepal (Table 5.1). It appears that no similar study was conducted previously in the eastern Terai and also no reports are available from the Terai in other regions of Nepal, however, the prevalences recorded in this study are in close agreement with the published reports from the central and western hills (Table 5.1).

In the present study, however, examination of the livers at the slaughter places showed much higher prevalences (an overall of 80.0% in buffaloes and 35.9% in goats) than those based on the faecal examination. It is well established that even in patent fasciolosis, the detection of the eggs in the faeces of infected animals depends on several factors such as sensitivity of the methods employed (Morel, 1987), host age and defecation and gallbladder evacuation rhythms, fluke egg-laying rhythms and sampling techniques (Horner, 1965a, b and c; 1967). It appears, therefore, that the prevalences recorded in the present study on the basis of faecal examination and those reported by the other workers using similar methods are almost certainly underestimates.

Table 5.1. A summary of the reports on prevalence of fasciolosis in ruminants from the eastern, central and western hills of Nepal.

Region	Species	Number sampled	Prevalence (%)	References
Eastern	Cattle	660* 60** 120	38-59 10-60 59	Morel (1985) Morel and Mahato (1987) Thakuri and Mahato (1990)
	Buffalo	660* 120 186	50-75 63 46	Morel (1985) Thakuri and Mahato (1990) Shrestha et al. (1992)
	Goat	120	23	Thakuri and Mahato (1990)
Central	Cattle	2026 60	42 51	Anon (1984) Oli et al. (1989)
	Buffalo	1599 60	61 68	Anon (1984) Oli et al. (1989)
	Goat	666 60	25 17	Anon (1984) Oli et al (1989)
Western	Cattle	NI 125	66 66	Lowcock (1982) Joshi (1988)
	Buffalo	NI 262	79 78	Lowcock (1982) Joshi (1988)
	Goat	NI 89	37 8	Lowcock (1982) Joshi (1987)

Note: * = number of animals monitored from February to July 1985; ** = number of animals monitored from January to December 1981; NI = not indicated.

In general, the prevalences in animals were higher in the mid altitude area followed by the low and the high altitudes. This trend was found to be associated with the prevailing farming practices in the study sites. While rice was the major crop in the mid altitude area, this was a minor crop in the low altitude area, and an altitude of about 1850 m was the upper limit of rice cultivation. A similar association between the prevalence of fasciolosis and rice area was also observed by Joshi (1988) in the western hills and Shrestha et al. (1992) in the eastern hills.

In the Terai, except for buffaloes in which the prevalence was lower in Bhantabari/Jhumka, no trends were discernible in the prevalence between the VDC areas surveyed. As there are many swamps and canals in Bhatabari and Jhumka, one would expect a higher prevalence of fasciolosis in animals in these areas; in fact it was higher in goats, although not significantly so. However, the farmers in these VDC areas appeared to be very conscious about treating their animals for fasciolosis, particularly buffaloes. Generally goats are not treated. It is likely, therefore, that most of the buffaloes were treated with fasciolicide before the sampling.

Stall-fed buffaloes in the hills had a significantly higher prevalence of fasciolosis than grazed ones. The reverse was the case for cattle, although, the difference was not significant. Most of the grazed buffaloes included in this study were from the high altitude area. It would therefore be expected that the high altitude buffaloes would have a lower prevalence while these from the mid and low altitudes were stall fed. The majority of cattle are grazed on fallow rice-fields and marginal land below 1850 m where there are abundance of *Lymnaea* habitats, therefore, a higher prevalence in them could be expected. Non-migratory sheep had a significantly higher prevalence of fasciolosis compared to migratory ones. Non-migratory sheep graze throughout the year on fallow rice fields and marginal land below 1850 m while migratory sheep have very limited access to such grazing.

In the hills, the highest prevalence was recorded in buffaloes followed by cattle, goats and sheep. However, in the Terai, the highest prevalence was found in cattle followed by buffaloes and goats, with an insignificant difference between cattle and buffaloes. The data of the longitudinal survey suggested that the monthly prevalences were generally higher in buffaloes than cattle. Higher prevalences in buffaloes followed by cattle and goats were also observed in the western hills by Lowcock (1982) and Joshi (1988; 1989). Similarly, Swarup and Pachauri (1987a) and Chaudhri, Gupta, Kumar, Singh and Sangwan (1993) recorded higher infection rates of *F. gigantica* in buffaloes than cattle in India. These workers suggested that this could be attributed to relatively higher susceptibility of buffaloes by virtue of their habitat and grazing behaviour in swamps. In the present study, however, the prevalence was higher even in stall-fed buffaloes which were fed mainly rice-straw, tree fodder and grass from rice-fields. This finding suggested that either there is a greater resistance to fasciolosis in cattle than buffaloes or the food provided to stall-fed buffaloes is the main source of infection with *Fasciola* spp.

The difference in prevalence between large (cattle and buffaloes) and small (goats and sheep) ruminants could be due to three reasons. Firstly, large ruminants tend to live longer than small ruminants and consequently they are exposed to infection for a longer time. Secondly, goats browse while cattle and buffaloes are basically grazers. This browsing behaviour is probably the major reason for the low prevalence in goats. Thirdly, the lower prevalences in small ruminants could be associated with the fact that most small ruminants succumb to acute fasciolosis caused by *F. gigantica* (Hammond, 1970).

The prevalences in all the species increased with age which is in agreement with Lowcock (1982), Swarup and Pachauri (1987a) and Pachauri, Yadav and Swarup (1988). This could be due to the longer exposure of older animals to infection and to carrying residual infections from previous years. Swarup and Pachauri (1987a) suggested that the higher prevalence in older female animals might be due to the relaxation of resistance at parturition or during lactation. Castelino and Preston (1979) found that in cattle, much of the increase in prevalence with age was in light infection category. Based on their findings, these workers suggested that there is an equilibrium between acquiring severe infections and the elimination of the infection thus causing a reversion to a light infection. In this study, a decline in the prevalence was seen in cattle over 11 years old. There was also some decline in the faecal egg counts in this age group of cattle. However, this was not the case for buffaloes, goats and sheep. Although no data on the fluke burdens in different age groups of cattle, goats and sheep are available, there was no evidence to suggest that these declined with age in buffaloes.

Generally, a higher prevalence was recorded in female animals than males. This higher prevalence in females was mainly due to the contribution of older animals. As most of the male buffaloes, goats and sheep are sold when they attain marketing age, a higher proportion of these animals were young (below 2 years). However, in cattle in which male to female ratio was nearly equal in all age groups,there was no difference in the prevalence between male and female.

5.2 Intermediate Host

In the present study, four *Lymnaea* spp. were found in eastern Nepal, namely, *L. auricularia* race *rufescens*, *L. auricularia sensu stricto*, *L. viridis* and *L. luteola*. However, doubt still exists as to whether snails identified as *L. viridis* might not be example of *L. andersoniana* which has been incriminated as intermediate host of *F. gigantica* in Sikim, India (Katiyar, Srivastava, Khera and Lepcha, 1983, cited by Prasad et al., 1987). Among the snails identified, *L. auricularia* race *rufescens*, *L. auricularia sensu stricto* and *L. viridis* were found to be natural hosts for *Fasciola* spp. Only these three species of snails were found in the middle and high hills where animals were found also to be infected with *F. hepatica* and the intermediate form. It would, therefore, be interesting to investigate whether any of these or all of these snail are the vectors for both *F. gigantica* and *F. hepatica* or even the intermediate form of *Fasciola*.

In 1973, Singh et al. (1973) collected snails from different regions of Nepal and found *L. auricularia* race *rufescens* and *L. viridis* to be widespread. Morel and Mahato (1987) reported that *L. auricularia* race *rufescens* and *L. luteola* (or possibly *L. viridis*) were responsible for the transmission of *F. gigantica* in the Koshi hills of eastern Nepal. However, in the present study *Fasciola.* spp. infection was not recorded in *L. luteola* which is in agreement with Kendall and Parfitt (1965).

While *Lymnaea* spp. were occasionally found in habitats which were not primarily suited to them, they usually occurred in such unsuitable habitats in very small numbers. The present study suggested that the optimum habitat conditions under which these snails occurred in large numbers were as follows:

- the water which is not much disturbed by animals,
- clear and clean water with an absence of red precipitate,
- permanent water 5-8 cm in depth, even in the dry season,
- slow- flowing water of a rate insufficient to dislodge the snails,
- a pH of 6.0-8.0,

- a temperature of 22-25° C,
- clay bottom,
- presence of a small number of aquatic weeds
- an abundance of algae.

From the present observations, it was not possible to ascertain the optimum range for the electro-conductivity of water, however, there was a significant positive correlation between this (20-240 μs) and snail density. Similarly, while there were indications that a high concentration of dissolved oxygen in water is favourable to the *Lymnaea* spp., the optimum range could not be determined. Although, a single habitat at an elevation of 2100 m was populated with a few *Lymnaea* spp., dense populations of these snails were found up to around 1600 m. Furthermore, there was a significant negative correlation between the percentage of water bodies harbouring *Lymnaea* spp. and the altitude ranges. The unfavourable condition with increase in altitude may be an indirect effect through temperature, pH, electro-conductivity and dissolved oxygen because these were found to be negatively correlated with altitude.

Rice-fields, in which almost all of the above conditions are available, provide ideal habitats for *Lymnaea* spp. particularly in the hills. The fields are frequently contaminated by dung from infected animals, thus ensuring the continuity of the life cycle of *Fasciola* spp. Therefore, any forage resource obtained from these ricefields could be a potential source for *Fasciola* infection.

Although, rivers, canals, fish ponds, ditches around drinking water places, seepage from canals, concrete tanks were all habitats of *Lymnaea* spp., road-side pools appeared to be the most important habitat in the Terai from the epidemiological point of view. These pools are daily contaminated with the faeces of animals which graze around them throughout the year. During the monsoon, the pools are connected with extensive areas of rice-fields and the infected snails spread over the fields.

The population of *Lymnaea* spp. was high during the dry period and declined with the onset of the monsoon. The population figures during the monsoon are almost certainly underestimates as abundant vegetation and high waters level hindered sampling. Furthermore, due to expansion of the habitat size, there was a dilution effect on the population density during the wet season. Snail egg masses and young snails were observed throughout the year, it would appear that there is no marked seasonality in reproduction. However, in the hills the reproduction and growth might have been retarded during November-March when the mean water temperature was below 20°C (Kendall and Parfitt, 1965).

It is well known that climate, especially temperature, has an important influence on the ecology of *Lymnaea* spp. However, it was not possible to determine the exact influence climate had on the population dynamics of snails under natural conditions. Considerable climatic differences can occur not only from season to season but also from one area to another area particularly in the hills. It could, however, be concluded that up to around 1700 m climatic conditions are conducive to the multiplication of the snail vectors of *Fasciola* spp. in eastern Nepal.

Laboratory observation showed that *Lymnaea* spp., particularly *L. viridis* and young *L. auricularia* race *rufescens* may survive in dry mud for at least a month. This supports the view of Singh et al. (1973) who, on the basis of field observations, stated that the aestivation period of *Lymnaea* spp. is about 5 months in Nepal. The implications of these observation are considerable. First, these snails may survive, and fasciolosis may occur, in areas where temporary water sources are used for watering stock or where seasonal swamp is used for dry season grazing. Secondly, present concepts of the use of molluscicides in the control of *F. gigantica* infection may have to be revised. If the snail vectors of *F. gigantica* were indeed solely aquatic, the strategic and most economical time for application of molluscicide would be in the dry season when the water volume was at its lowest. Since it is clear that *L. viridis* and *L. auricularia* race *rufescens* may aestivate for months in dry mud, the use of a molluscicide at this time might leave significant numbers of snails unharmed.

5.3 Epidemiological Cycle

5.3.1 *Epidemiological cycle in the hills*

Redial infections were not found during February-April which could be the combined effects of low temperatures and low rainfall. Although, animals contaminated fallow rice-fields, possibly faecal pats dried quickly, preventing the release of *Fasciola* eggs and subsequent contamination of adjacent habitats because of only light showers during this period (less than 5 days/month). Furthermore, the low water temperatures which continued until March, may have had an adverse effect on the hatching of eggs and the development of subsequent larval stages in the snails even where there were contamination of the habitats. Redial infections were first seen in May, coinciding with the pre-monsoon showers. Except for a gap in September, which was possibly due to the lower number of the snails examined, the infections continued until January. The pattern which emerged suggested that there was continued contamination of the habitats, because most of the springs which fed these habitats are also used as drinking places for animals. The first mature (redial and cercarial) infections were detected in May and these continued until February. The absence of immature infections from February to April and mature infections in March and April suggested that there was no overwintering of the infections in snails. A closely similar pattern of the larval infections in snails was also observed by Morel and Mahato (1987) in the same area.

Trends of the prevalence seen in cattle and goats showed that the majority of these animals were infected while grazing in the fields during May-June and to some extent in July, especially in the low altitude area. Infections became patent in August-September reflecting the high prevalences during this period. After rice planting in July, grazing in the fields was stopped until the harvest during the mid to the end of October. Grazing in the fields after the harvest accounted for the high prevalences of chronic fasciolosis during December-March. Although, the incidence of fasciolosis in the tracer zebu calves was recorded almost every month (except for November), the pattern was very similar to those of the prevalences in cattle and goats.

In buffaloes, however, the trends were slightly different from those in cattle and goats which reflected the difference in their feeding systems. The high incidence in the tracer buffalo calves was observed during October-November and again during February-April. The prevalence also followed a very similar pattern, although a slight rise was seen in August. As stated earlier, buffaloes were mainly stall-fed. With the onset of the monsoon, they were provided with rice-straw, maize plants and grass cut and carried from rice-fields. From the mid monsoon, rice-straw was replaced by other crop by-products, such as maize stover, maize husks and millet straw. Although, grass from the fields was provided in limited quantities during the whole monsoon period, it constituted a major part of the diet from the mid monsoon onward. After rice harvest, rice-straw supplemented with tree fodder is the principal food until April-May. These observations suggested that grass from the fields was the source of the metacercariae causing infections which became patent from August to November. The metacercariae which were encysted on rice-straw contributed for the high prevalence during February-April. Patent infections in the tracer buffalo calves and immature flukes were recorded throughout the dry period until April-May. Supporting the findings of Joshi (1987), this suggests that metacercariae may survive in rice-straw for a considerable period of time.

In this context, it must be pointed out that although the prevalence and possibly also the fluke burdens are higher in buffaloes than other animals, their role in the continuation of the epidemiological cycle by contaminating pastures/habitats with fluke eggs is least important in the hills of eastern Nepal. Cattle, goats and sedentary sheep appear to be the main source of such contamination. Small ruminants, therefore, should also be dosed in any programme which aims to control fasciolosis by reducing habitat/pasture contamination.

5.3.2 *Epidemiological cycle in the Terai*

In the Terai, although climatic conditions were suitable for the hatching of eggs and the development of subsequent larval stages in the snails, no redial infections were recorded during August-November. This may have been due to the multiplicity of water sources available during these months, with the consequent reduction in the number of stock grazing adjacent to the habitats under study, and to the monsoon rains washing away *Fasciola* eggs coming from those animals still grazing these sites. A resurgence of redial infection was detected in December, probably from eggs hatching in November when the animals again had free access to these sites following the rice harvest. By then the dry winter season had started preventing hatching of the eggs until February. Mature infections in snails were seen every month with the highest rate in September. There was then a continuous decline in the infection rates until March, which suggested that the snails with mature infections were dying and there had been little or no further infections. However, a few mature infections over-wintered in the snails.

In general, cattle and buffaloes showed more or less similar trend of the incidence and prevalence. In the majority of animals, the infection became patent during July-August, suggesting that these had ingested the metacercariae during April-May while grazing lush green grass available only near the snail habitats during this period. With the onset of the monsoon rains, most of the areas adjacent to *Lymnaea* habitats were under water and thus unaccessible for grazing. On the other hand, enough grass on marginal uplands became available. During the post monsoon period (autumn), when water levels in the habitats dropped, the adjacent areas again became available for grazing. The metacercariae ingested by animals during this period probably accounted for the high infection rates during December-February (possibly infection rates had been higher as some animals were dosed with a fasciolicide by Government Animal Health Workers after the November sampling). A further contribution to this rise could be attributed to grazing in rice-fields following the harvest in November.

Goats showed a different prevalence pattern from cattle and buffaloes The prevalence was high from March to September with peaks in March and June. As mentioned earlier, the prevalence of fasciolosis in goats in the Terai was studied in the Letang VDC area only. While goats generally grazed with cattle and buffaloes throughout the year in other VDC areas, these were grazed in a nearby forest from

June/July to October/November and on road sides and fallow rice-fields during December to May. The high prevalence observed during March to June, therefore, appeared to be due to infections acquired from road sides and rice-fields.

PART TWO

PATHOGENESIS STUDIES

CHAPTER SIX

LITERATURE REVIEW

PATHOGENESIS OF FASCIOLOSIS WITH SPECIAL REFERENCE TO ITS EFFECTS ON PRODUCTIVITY OF RUMINANTS

6.1 Introduction

There is a large amount of literature published on the pathogenesis of *F. hepatica* infection. However, relatively little comparable information has been published on *F. gigantica* infection, despite its considerable economic importance in Asia and Africa. Except in a few instances, there is no reason to suppose that much of the work on *F. hepatica* does not also apply to *F. gigantica*. Extensive reviews on various aspects of the pathogenesis and pathology of fasciolosis caused by *F. hepatica* have been provided by Dawes and Hughes (1964), Pantelouris (1965) and Sinclair (1967). Sewell (1966) reviewed the pathogenesis of acute and subacute fasciolosis caused by *F. gigantica* in zebu cattle and discussed similarities and differences between syndromes caused by *F. hepatica*. Boray (1969) reviewed experimental fasciolosis caused by *F. hepatica* in Australia. Different aspects of fasciolosis in ruminants, including the syndrome caused by *F. gigantica* was reviewed by Malek (1980) and Boray (1985). In this chapter, some aspects of pathogenesis which are relevant to the present study together with the associated production losses which occur in fasciolosis are briefly reviewed.

6.2 General aspects

Fasciolosis has been described as having three different syndromes, namely acute, subacute and chronic since the clinical signs vary in severity according to the number of metacercariae ingested (Sewell, 1966; Hammond, 1970; Pullan, Sewell and

Hammond, 1970). Grazing behaviour of the animals and different husbandry systems were thought to be contributory factors (Sinclair, 1969). Sheep are more prone to acute fasciolosis perhaps because they frequently graze in snail habitats. Goats may avoid the disease because of their preference for browsing on shrubs. The grazing habits of cattle may also tend to diminish the rate of intake of metacercariae as they rarely suffer from the acute disease (Taylor, 1964).

Sudden death without clinical signs is the characteristic feature of acute fasciolosis and it has been observed in sheep 7 to 8 weeks after *F. hepatica* infection with 5000 metacercariae (Ross, Dow and Todd, 1967; Boray, 1967; Roberts, 1968). Acute fasciolosis is sometimes also called parenchymal fasciolosis as it occurs at the time that the migrating flukes are causing severe parenchymal hepatic damage in cattle and sheep resulting in haemorrhage, necrosis and interstitial fibrosis (Reid, Dargie, Murray, Armour, and Over, 1973). Subcapsular haemorrhage in the liver and blood tinged fluid in the abdominal cavity were characteristic features. Death during acute fasciolosis is due to failure in liver function, with anaemia a less prominent feature (Boray, 1967).

Ross et al. (1967) described subacute fasciolosis in sheep between the 12th and 20th week post infection with 800 to 1500 metacercariae of *F. hepatica*. At this time 30 to 60 percent of the adult fluke were in the bile ducts. As a result of mechanical tissue damage there was extensive haemorrhage. Due to tissue reaction, prolonged migration of flukes leads to further mechanical lesions. Localized haemorrhagic plaques and numerous migrating fluke tracts in the subcapsular parenchyma are the most distinct lesions found at necropsy (Ogunrinade, 1983; 1984). Sewell (1966) observed that jaundice may be a marked feature in cattle in the later stages of sub-acute fasciolosis caused by *F. gigantica* while a rapid weight loss was observed after 8 weeks of infection.

The parenchymal hepatic damage results in impairment of liver functions such as the metabolism of proteins, carbohydrates and fat, synthesis of protein and secretion and excretion of bile (Doxey, 1983). The homeostatic function of the liver is also affected (Harvey, 1962). Liver function is impaired in acute and subacute fasciolosis indicated by extension of bromosulphthalein retention time (Sinclair, 1966; Boray, 1967; Roberts, 1968; Pullan et al., 1970).

Chronic fasciolosis is very important as it is much more common than acute and sub-acute fasciolosis. It runs a prolonged course and gives rise to many subclinical infections (Pullan et al., 1970), with the affected animal surviving for a long time and experiencing long term ill effects. Ross et al. (1967) encountered chronic fasciolosis in sheep with 200 or more adult *F. hepatica* in the bile ducts. Hammond (1973) could reproduce this syndrome in sheep with as few as 60 *F. gigantica* metacercariae. Chronic fasciolosis correlates with the arrival of the flukes in the biliary system (Sinclair, 1969). Hammond (1970) and Reid et al. (1973) demonstrated hyperplastic cholangitis in the biliary stage of fasciolosis. It appeared to be a result of the severe trauma inflicted on the biliary mucosa by the presence of flukes, with extensive erosion and necrosis. However, the reaction in the bile duct epithelium is more severe in cattle in *F. gigantica* infection and is characterised by the occurrence of calcification (Hammond, 1970; Ogunrinade, 1983). Characteristic fibrotic changes in the liver parenchyma in the chronic stages appear to be a form of repair mechanism since collagen formation follows tissue destruction (Murray and Rushton, 1975). The major effects of chronic fasciolosis are loss of weight, anaemia and hypoalbuminaemia (Sewell, 1966; Hammond, 1970; Ogunrinade, 1983).

Berry and Dargie (1976) compared the course of the disease, firstly in sheep given the same number (1,000) of *F. hepatica* metacercariae and fed a ration containing 6% or 13% crude protein; secondly in chronically infected sheep transferred from a high to a low protein diet. Results of the first experiment showed that, sheep on the lower protein diet lost weight more rapidly, developed anaemia and hypoalbuminaemia and died earlier than their better fed counterparts. In the second experiment, it was found that the disease developed faster in infected sheep when they switched from a high to low plane of nutrition. In another experiment (Dargie et al., 1979), sheep infected with *F. hepatica* were given a diet of hay or hay with concentrate. After the sixth week the reduced appetite and body weight loss were more marked in the group given the diet of hay alone. Sheep on the diet of hay alone became moribund by week 14, whereas those given hay with concentrate survived until week 20. It seems likely, therefore, that the change from better to poor quality diet reduced the capacity of the infected sheep to withstand the pathogenic effects of the infection.

6.3 Clinico-pathological aspects

6.3.1 *Anaemia*

Anaemia has been described as the most characteristic sign of subacute and chronic fasciolosis. Hammond (1970) noticed a decrease in packed cell volumes, haemoglobin concentrations and RBC counts in sheep infected with *F. hepatica* beginning 2-4 weeks after infection. A decrease in RBC counts and haematocrit values was observed at both 6 and 10 weeks after infection respectively in the same species of animal infected with *F. gigantica* (Kadhim, 1976). Ogunrinade and Bamgboye (1980) reported that in sheep infected with *F. gigantica*, anaemia occurred 10 to 12 weeks after infection, at the time when adult flukes were present in the bile ducts. In acute infection in sheep, however, the onset of anaemia was noticeable by the sixth week and became pronounced by the eighth week of infection (Ogunrinade, 1984).

Although macrocytosis and hypochromia have been observed in some studies, the anaemia in fasciolosis is generally normocytic and normochromic (Sinclair, 1964; Sewell, 1966, Hammond, 1970; Ogunrinade, 1983; Swarup, Pachauri and Mukherjee, 1987; Chaudhri, Mandokhot, Gupta and Yadav, 1988; Wiedosari and Copeman, 1990). The severity of anaemia in *F. gigantica* infection has been associated with fluke burden and stage and duration of infection (Ogunrinade and Bamgboye, 1980). Hawkins (1984) suggested that haemoglobin concentration and packed cell volume may be used to predict the fluke burden.

The aetiology of anaemia in fasciolosis has been studied for many years and a number of hypotheses have been advanced by different authors. Stephenson (1947) observed intact RBCs in the caecal contents of flukes and Todd and Ross (1966) concluded by spectroscopic examination that the caecal contents of *F. hepatica* contained haemoglobin breakdown products. The amount of blood loss caused by *F. hepatica* was first measured by Jennings, Mulligan and Urquhart (1956) using ^{32}P-labelled red cells. They concluded a figure of 0.2 ml of blood loss per fluke per day. However, when other red cell labels (^{51}Cr and ^{59}Fe) were used it was found that the red cell loss per fluke was about 0.5 ml/day (Holmes, Dargie, MacLean and Mulligan, 1968a). In heavy infections this may represent a considerable loss of RBCs.

Lapage (1968) stated that the production of a toxin by *F. hepatica* may have a deleterious effect on erythropoiesis, while Sinclair (1962; 1964) assumed that there is some interference by toxins in erythropoiesis or a shortening of the life span of cells. Other possible factors such as an inadequate supply of iron, copper, cobalt, vitamins, proteins and disturbances in protein and iron metabolism, that might affect erythropoiesis were pointed out by Sinclair (1964; 1965).

Proline, an amino acid released in large quantities by adult *F. hepatica* has been shown to cause increased red blood cell lysis; therefore, its role in the development of anaemia has also been considered (Isseroff, Spengler and Charnock, 1979). However, Ogunrinade and Makinde (1988) showed that infusion of 700 μmol proline/rabbit/day for 14 days did not significantly alter the half-life of ^{51}Cr labelled erythrocytes nor did it interfere with plasma clearance and utilization of ^{59}Fe. Consequently, they concluded that proline did not interfere with haemoglobin synthesis or induced haemolysis in rabbits at the test dose level.

Using ^{51}Cr labelled RBCs, Dargie, Holmes, MacLean and Mulligan (1968) showed loss of RBC and plasma into the gastrointestinal tract in *F. hepatica* infected rabbits and the same phenomenon also occurred in sheep (Sewell, Hammond and Dinning, 1968). These latter workers considered that the primary cause of the anaemia is loss of blood into the bile, though there may be an associated haemolysis as a result of destruction of poikilocytes and immature erythrocytes in the peripheral blood in severe chronic fasciolosis.

6.3.2 *Eosinophilia*

An increase in the number of eosinophils in tissue lesions and/or blood is a common feature following helminth infections including fasciolosis (Butterworth,1984; Yoshimura, 1989). It was considered by Furmaga and Gundlach (1978) to relate to the "cell-participation-defence process" against the parasites. In calves infected and reinfected with *F. hepatica* they showed a many-fold increase in the eosinophil counts as compared with the uninfected controls. Similarly, Hammond (1970) showed that as few as 10 *F. hepatica* caused a pronounced eosinophilia in sheep. He also demonstrated that calves, experimentally infected with *F. gigantica* had very marked bi-modal eosinophilia which tended to parallel the total leucocyte counts. Most of the calves had gradual rises, which were evident as early as the first week after infection.

In rabbits infected with 500 or 1,000 *F. hepatica* metacercariae, Bolbol (1975) observed eosinophilia up to seven weeks after infection, and Furmaga, Gundlach and Sobieszewski (1974) observed a similar response in rabbits and sheep during the acute phase of invasion (3-10 weeks) by the fluke. Thereafter, the count dropped to approximately normal levels by week 11. Akahane (1975) studied the response of heavily and lightly infected rabbits and concluded that the eosinophilia appeared earlier in the former. It is evident from various studies, however, that eosinophilia of the peripheral blood is not an index of the intensity of the infection as no consistent correlation between the eosinophil count and the fluke burden has been demonstrated (Hammond, 1970; Ogunrinade and Bamgboye, 1980).

The eosinophilia of helminth infections has been demonstrated to be a T lymphocyte-dependent event by Basten and Beeson (1970), Walls and Beeson (1972) and Walls, Bass and Beeson (1974). In their classical experiments, these workers demonstrated that after intravenous injection of *Trichinella spiralis* muscle stage larvae in rats, a sharp burst of eosinophilia occurred, reaching a peak 10 days after injection. Depletion of T lymphocytes by neonatal thymectomy, thoracic duct drainage or treatment with anti-lymphocyte serum ablated this response and, after transfer of lymphocytes from infected animal to normal syngeneic recipients an enhanced and accelerated response was observed. A similar T lymphocyte-dependent eosinophilia has been noted in several other helminth infections, including schistosomiosis (Colley, 1972; 1974) and ascariosis (Walls, 1976). However, not all helminth associated eosinophilia are T lymphocyte-dependent; for example, athymic (nude) rats are capable of mounting an eosinophilic response to *Ascaris suum* (Pritchard and Eady, 1981) and *F. hepatica* (Doy and Hughes, 1982) infections.

In addition to an increase in circulating eosinophils, helminth infections commonly elicit high IgE responses (Jarrett and Miller, 1982); and, during repeated or prolonged infections, there may be evidence of extensive IgE mediated reactions (immediate hypersensitivity reactions) to helminths or helminth products (Jakubowski and Barnard, 1971; Bryceson, Warrell and Pope, 1977; Enzenauer and Yamaoka, 1982). However, there is no evidence that the two processes are causally linked, whereas the subsequent influx of eosinophils into the site of immediate hypersensitivity reactions is directly dependent on IgE-mediated reactions. For example, Dessein, Parker, James and David (1981) have shown that suppression of the IgE response of rats to *T. spiralis* infections, by treatment of neonatal animals with anti-epsilon serum before infection, causes a marked reduction in local eosinophil accumulation around the encysted larvae, but has relatively little effect on the development of blood eosinophilia. In addition, there is no direct relationship between the development of eosinophilia in different inbred strains of mice and their capacity to mount an IgE response. It would appear, therefore, that the eosinophilia and the IgE responses seen in helminth infections are separate phenomena.

Eosinophils have been found to damage *Schistosoma mansoni in vitro*, and circumstantial evidence indicates that they may play a role in resistance of rats to *F. hepatica* (Davies and Goose, 1981; Burden, Bland, Hammet and Hughes, 1983). Milbourne and Howell (1990) examined the qualitative and quantitative cellular changes in the peripheral blood and bone marrow of resistant (rat) and susceptible (mouse) hosts of *F. hepatica*. In infected rats, the eosinophilia was not significantly raised until after day 21, but levels remained high for the remainder of the experiment. Infected mice showed a significant rise in eosinophil numbers after day

7 but levels dropped by day 27. That eosinophils are involved as cellular effectors in resistance to infection in rat is suggested by changes in their numbers in peripheral blood during infection. More direct support comes from the observation that rat eosinophils adhere to and kill juvenile flukes in the presence of infected rat serum (Davies and Goose, 1981).

Several workers have examined the steps leading up to cytotoxicity of eosinophils in helminth infections including *S. mansoni* in humans (David, Butterworth and Vadas, 1980), *S. mansoni*, *Trichinella spiralis*, and *Nippostrongylus brasiliensis* in rats (McLaren, McKenzie and Ramaiho-Pinto, 1977), and *F. hepatica* in rats (Davies and Goose, 1981). All seem to have come to a consensus view on how this operates. Eosinophils adhere to the surface of the parasite and then degranulate, releasing peroxidase either directly onto the parasite and causing damage or into cytoplasmic vacuoles which then discharge onto the parasite.

6.3.3 *Changes in plasma/serum protein levels*

Chronic fasciolosis is associated with profound alterations in the plasma/serum protein profile. Serum protein estimations were carried out by Hammond (1970) on calves infected with three levels of *F. gigantica* metacercariae. The total serum protein concentration increased at all three levels of infection. The highest concentrations were seen at 13-16 weeks after infection. There were no significant differences from controls in albumin concentrations in the calves infected with 500 metacercariae but the concentration fell in the calves which were infected with 1000 and 2000 metacercariae. However, Sewell (1966) observed that although the total serum protein levels remained within normal limits the albumin-globulin ratio fell considerably, in one of the two calves infected with 300-500 *F. gigantica*, whereas in the other the total serum protein concentration fell below normal by about 17 weeks after infection, but the albumin-globulin ratio remained fairly constant. Weinbren and Coyle (1960) also reported variations in the serum protein levels in naturally infected and control cattle. They observed a highly significant fall in the albumin-globulin ratio with an increase in the γ-globulin concentration, but no significant change in the total serum protein concentration. In a study in Bangladesh, a similar alteration in serum protein constituents was observed in natural *F. gigantica* infection in goats (Nooruddin, Samad and Rahman, 1982) and buffaloes (Kumar, Maru and Pachauri, 1982). In naturally infected buffaloes in India, however, Chaudhri, Mandokhot, Gupta and Yadav (1988) found significantly lower serum protein levels.

The changes in the serum proteins in *F. hepatica* infections have been studied by many workers, and are similar in cattle and sheep. The initial hyperglobulinaemia, which is due mainly to an increased γ-globulin concentration, is accompanied later by hypoalbuminaemia which tends to persist during the presence of the adult flukes in the bile ducts for both cattle (Ross, Todd and Dow, 1966) and sheep (Sinclair, 1962; Roberts, 1968).

Studies using radio-labelled albumin and IgG_2 in cattle and sheep with a patent *F. hepatica* infection confirmed that plasma protein loss is the major cause of changes in plasma protein profile with increased production of immunoglobulin playing a secondary role (Nansen, Eriksen, Simensen and Nielsen, 1968; Holmes, Dargie, MacLean and Mulligan, 1968b). During the patent period of infection in the bile ducts, turnover rates and faecal excretion of radioactivity increased markedly, showing that there was a loss of protein through the inflamed bile ducts in rabbits (Dargie and Mulligan, 1971). A substantial part of this loss is probably associated with blood sucking activities of flukes but there may also be a continued passage of plasma after the flukes have finished their blood meal.

6.3.4 *Changes in serum mineral concentrations*

The possible effects of fasciolosis upon serum mineral concentrations has not been examined in detail. Sinclair (1962, 1965), studying the clinical pathology of ovine fasciolosis caused by *F. hepatica*, observed that there was a fall in serum magnesium levels during the later migratory phase, and when the flukes reached the bile ducts, the serum calcium level began to fall. There was a gradual fall in serum iron values followed by a sharp fall in total RBC counts suggesting that the decrease in iron content was secondary to anaemia. However, there is also a report of no appreciable deviation from normality in mineral levels in *F. hepatica* infected sheep (Sykes, Coop and Rushton, 1980).

In buffaloes naturally infected with *F. gigantica*, Haiba, El-Rawii and Osman (1964) observed a significant decrease in the serum and bile calcium levels, but not in phosphorus and magnesium, and significantly higher bile magnesium levels. Chaudhri et al. (1988) reported that *F. gigantica* infection in buffaloes caused a significant decrease in serum iron and calcium as well as phosphorus. However, Sewell (1966) reported that none of his 4 infected cattle showed the drop in serum magnesium concentration and only one animal showed a reduction in serum calcium concentration at the end of the experiment.

Haiba et al. (1964) suggested that a non-diffusible part of the blood serum calcium is probably attached to the serum albumin, a decrease in the latter protein may thus result in a subsequent decrease in the serum calcium level. They further suggested that the significantly high bile magnesium levels in infected animals can be accounted for by the generally known inverse relationship between the calcium and magnesium levels in both healthy and diseased animals.

6.3.5 *Changes in serum enzyme concentrations*

The main feature of fasciolosis is increased plasma/serum levels of liver enzymes. The elevation of liver enzymes, such as aspartate aminotransferase (AST/GOT), ornithine carbamyl transferase (OCT), sorbitol dehydrogenase (SD), glutamate dehydrogenase (GLDH) and γ-glutamyl transferase (GGT) have been observed in fasciolosis in cattle (Hammond, 1970; Ogunrinade, 1983; Suhardono, Widjajanti, Stevenson and Carmichael, 1991), in buffaloes (Kumar, Maru and Pachauri, 1982; Swarup and Pachauri, 1987b; Chaudhri et al.,1988), in sheep (El Samani, Mahmoud, Fawi, Gameel and Haroun, 1985; Sykes, Coop and Robinson, 1980) and in goats (Hughes, Treacher and Harness, 1973; Haroun, El Sanhouri and Gameel, 1988).

The presence of excess amounts of liver specific enzymes in the plasma or serum is an indication of liver damage. The rate of release of an enzyme from cells depends on the nature of the tissue damage, the binding of the enzymes to cell components and the original concentration of enzyme in the damaged organ (Ford, 1965). The value of any enzyme assay is governed by its sensitivity, liver specificity and the stability of the enzyme in the plasma (Sykes et al., 1980).

Sewell (1967) suggested that, while an increase in the serum GLDH activity is not specific for fasciolosis, it is much more specific for liver damage than an increase in aspartate aminotransferase, which is more pronounced in myopathies than in parenchymal liver damage. Anderson, Berrett and Patterson (1978) tested 10 different enzymes as indicators of liver injury in calves experimentally infected with *F. hepatica* and found that the plasma GLDH and GGT activities were the most sensitive indicators of liver damage in fasciolosis. Since GLDH is a mitochondrial enzyme from the liver parenchyma, the elevated enzyme levels indicate an early acute disease and their levels decline as the fluke reach maturity and enter the bile ducts. On the other hand GGT which originates in the bile canaliculi of the liver reaches peak plasma levels as the flukes enter the bile ducts. In a comparison of various liver function tests in the cattle which were affected with different hepatobiliary diseases, West (1991) showed that both GLDH and GGT assays were 72.7% sensitive in fasciolosis, while their specificity varied from 83 to 100% and 50 to 100% respectively, being lowest in infectious conditions. It can be concluded, however, that in the absence of other data, elevated plasma or serum GLDH or GGT levels are sensitive indicators of the disease in its acute and subacute or chronic phase respectively and can be used for the field diagnosis of acute fasciolosis and for identifying the effective removal of the parasite from the host.

The measurement of SD in serum is a useful adjunct to the measurement of GLDH and OCT. Hawkins (1984) observed a number of peaks of SD activity, when the majority of flukes were in the bile ducts. In heavy fluke burdens continuation of the migrating phase beyond the normal prepatent period is thought to be a contributing factor to the continuation of increased plasma concentration of enzyme.

Sinclair (1970) suggested that fibrosis of the liver around the bile duct caused by an immune response result in the subsequent release of SD.

6.4 Effects on productivity

6.4.1 *Mortality*

Fasciola spp. are not generally considered as being responsible for disease epidemics, but rather come under the category of "production disease", causing reduced productivity without obvious clinical signs (Dargie, 1987). Nevertheless, outbreaks of clinical disease resulting in death occur even in developed countries where the prevalence of fasciolosis are generally low (Boray, 1969; Chick, Coverdale and Jackson, 1980).

Depending on levels of infection, mortalities do occur. This could occur in endemic areas due to the presence of infected intermediate hosts throughout the year (Preston and Castelino, 1977). This scenario is exacerbated in the dry season when animals are grazed in marshy areas. Kendall (1954) reported that the husbandry of sheep or goats proved impossible in many areas in Pakistan, owing to the occurrence of acute form of fasciolosis with a high rate of mortality.

6.4.2 *Liver condemnation*

For aesthetic reasons, all fluke infected livers are either trimmed or totally condemned. This forms an appreciable loss to the meat industry and the scale of the problem caused by *Fasciola* spp. is normally and misleadingly underestimated from liver condemnation rates. These have fallen dramatically in last 10 years in some countries, e.g. in the United Kingdom and Ireland from 35% and 75% to 6% and 38% respectively for cattle livers and to even lower levels (3% and 8% respectively) for sheep livers (Blamire, Goodhand and Taylor, 1980; Hope Cawdery, 1984). By contrast, according to the American Association of Veterinary Parasitologists (1983), approximately 1.5 million cattle livers are condemned as fluke-infected each year in the USA mainly in the Gulf Coast and Western States, but rates are rising due to increased use of irrigated land (Malone, Loyacano, Armstrong and Archbald, 1982).

6.4.3 *Body weight and composition*

The major effect of fasciolosis is considered to be that of reducing live weight or impairing growth. In experimental infections, the severity of weight loss was shown to be positively associated with the number of metacercariae administered and additionally influenced by the nutritional status of the host (Berry and Dargie, 1976). However, the fluke burden required to adversely affect weight or growth is not well established. One report suggests that weight gain is significantly depressed in sheep by as few as 45 adult *F. hepatica* (Hawkins and Morris, 1978). However, many studies have shown that burdens in excess of 350 *F. hepatica* cause severe weight loss and death in adult sheep (Sinclair, 1962; Boray, 1969; Berry and Dargie, 1976; Hawkins and Morris, 1978; Hawkins, 1984). Between these extremes there is work demonstrating the adverse effect on growth of burdens ranging from 87 to 230 adult flukes (Sykes, Coop and Rushton, 1980). The effective weight loss was less than 30 g/week with 45 flukes and 130 to 300 g/week in association with burdens of 87 to 500 adult flukes.

In cattle, there are conflicting views on the effects of fasciolosis. Housed and well-fed animals infected with *F. hepatica* had good growth rates (0.6 kg/week) even when infected with up to 440 adult parasites (Burden, Hughes, Hammet and Colis, 1978), but reduced weight gains and severe weight loss resulted when calves were maintained in paddocks during late autumn (Reid, Doyle, Armour and Jennings, 1972). There are reports that substantially lower numbers of *F. gigantica* affect growth rate in cattle (Sewell, 1966; Hammond, 1970). Over a period of eight and a half months of study on 39 Fulani zebu cattle experimentally infected with varying levels of *F. gigantica*, Sewell (1966) found that each fluke reduces the animal's liveweight gain by about 7 ounces (0.198 kg)/year. Although naturally infected cattle may appear emaciated, other conditions such as malnutrition and intercurrent diseases are likely to make the assessment of body weight loss difficult.

The observed depressed body weight gain is likely to be a consequence of impaired feed conversion efficiency, since the level of food intake remains unaffected at low level of infection (Hawkins and Morris, 1978). Reduced protein, fat and cellulose digestion may be associated with fasciolosis as shown by Chubaryan (1964) in *F. hepatica* infection in sheep and could account for the reduced rate of liveweight gain observed in these animals (Sinclair, 1967). There is also a clear evidence from one study that in sheep even relatively small burdens of *F. hepatica* (< 100 flukes) are capable of reducing body fat, protein, and energy deposition, and the efficiency with which metabolisable energy is used for growth (Sykes et al., 1980).

The possible effect of fasciolosis on body composition requires study particularly since in the terminal stages, polydypsia, increased water retention and ascites are notable features (Dargie, 1987). Over a period of 7 months study, Hammond (1973) found that the mean terminal liveweight of 10 sheep, each infected with 60 *F. gigantica* metacercariae was 5.6% less than the controls. When the sheep were slaughtered at 21-22 months of age, the dressed carcasses of the uninfected controls were 6.7% heavier and graded better than the infected sheep because of a higher proportion of fat.

6.4.4 *Milk production*

Data on the effects of fasciolosis on milk production and quality are extremely scarce and virtually all relate to *F. hepatica* indicating significant positive effects, following drenching. Depressions of milk yield ranging from 90-300 kg/lactation have been reported in cows infected with *F. hepatica* (Horschner, Hennings, Verspohl, Averbeck and Boch, 1976; Randell and Bradley, 1980). Reductions in total solids in milk resulting in a lower quality premium payment were also attributed to *F. hepatica* infection in cows (Black and Froyd, 1972). A study by Oostendorp and Over (1985) showed depressed yields ranging from 6.7 to 10.7%. After correcting the milk yield on a comparable milk fat content of 4%, the reduction was 13.3%. However, there is a report showing minimal effects on milk yield in cattle infected with *F. hepatica* (Hope Cawdery, 1984).

Effects on milk yield in sheep are not so straightforward since in many countries, sheep are not normally milked for domestic or commercial purposes. The effect on milk production is therefore measurable by decrease in liveweight gain of lambs born of infected dams. Lower live weight gain in lambs born of *F. hepatica* infected dam was noted (Sinclair, 1972; Reid and Armour, 1978).

6.4.5 *Wool growth and quality*

Roseby (1970) recorded a decrease in wool weight of between 20 and 30% from six weeks post infection with 400 to 500 *F. hepatica* metacercariae, although fibre strength and wax content of the greasy wool were not found to be affected. On the other hand, Edwards, Al-Saigh, Williams and Chamberlain (1976) observed poor wool quality, in terms of fibre length and diameter as well as staple length, in sheep given as few as 100 *F. hepatica* metacercariae. The rate of wool growth was also shown to be significantly depressed in association with *F. hepatica* infection (Reid and Armour, 1978; Hawkins and Morris, 1978; Hawkins, 1984). In *F. gigantica* infections, Hammond (1973) observed 10.2% reduction in the mean weight of unclipped air-dried skins for the group of sheep which were infected with 60 metacercariae. The mean weight of fleece-wool per infected sheep was 4.75 lb $\pm$ 0.35 compared to 5.40 lb $\pm$ 0.84 per control sheep.

In a review, Dargie (1987) pointed out that these effects of fasciolosis on wool growth and quality (a) are associated only with the presence of adult parasites, suggesting that they are not a reflection of liver damage; (b) can be brought about by as few as perhaps 30 adult *F. hepatica*, and hence, in the absence of any major effect on feed intake; and (c) are closely and directly related to the size of the fluke burden (e.g. between 23% and 50% in association with burdens of around 45 and 350 flukes, respectively). Finally, the quality of wool, as assessed by fibre diameter, length, strength, and colour may also be compromised by *F. hepatica* infection, resulting in lower grading and revenue to the farmer.

Reduced voluntary food intake and feed conversion efficiency were found to be the probable cause of the depressed rate of wool growth detected in sheep infected with *F. hepatica* (Roseby, 1970; Hawkins and Morris, 1978). However, there could also be an hormonal explanation for changes in wool quality. Wool may become tender with increased adrenocortical activity (Panaretto, Chapman, Downes, Reis and Wallace, 1975), which may result from hepatic dysfunction (Roseby, 1970).

6.4.6 *Reproductive efficiency*

F. hepatica infections reduce conception and pregnancy rates in cattle and increase the age at puberty (Oakley, Owen and Knapp, 1979; Foreyt, 1982; Malone et al., 1982). Reproductive loss in mature cows and replacement heifers ranged from 6-12% (Simpson, Kunkle, Courtney and Shearer, 1985), 4.8-20% (Oostendorp and Over, 1985) and 7-11% (Bohrender, 1988). In India, Kumar and Sharma (1991) observed that *F. gigantica* infection was one of the contributory factors responsible for infertility due to anoestrus and repeat-breeding conditions in rural cows. In sheep, studies in heavily infected ewes have demonstrated the occurrence of abortion and stillbirths in association with fasciolosis caused by *F. hepatica* (Sinclair, 1972; Hope Cawdery, 1976). There are also indications of reduced conception, pregnancy and lambing rates (Crossland, Johnstone, Beaumont and Bennett, 1977).

It seems unlikely that the reduced conception and pregnancy rates in *Fasciola* infections arise from nutritional stress *per se*, since although foetuses from ewes kept on a 25% maintenance ration were smaller and developmentally retarded, embryo survival was unaffected (Parr, Cumming and Clarke, 1982). The failure to maintain pregnancy and to show oestrus cycles of normal length or frequency may arise from: reduced amounts of steroids reaching target organs, e.g. uterus; release of prostaglandins in response to inflammatory reactions in damaged tissues in sufficient quantities to induce luteolysis (Fredriksson, Kindahl and Edqvist, 1985); and increased suckling frequency arising from the additional stress of parasites superimposed on a poor plane of nutrition, leading to prolonged anoestrus (Loudon, McNeilly and Milne, 1983).

6.5 Effects on feed intake and utilisation

6.5.1 *Feed intake*

Reduced feed intake is often quoted as being an important source of production loss in parasitised animals. In *F. hepatica* infections of growing lambs (Hawkins and Morris, 1978; Sykes et al., 1980), pregnant ewes (Sinclair, 1972), and adult wethers (Sykes et al., 1980), feed intake is not measurably depressed by pre-patent infections except when these resulted in acute or subacute disease. Intake is however depressed in association with adult flukes and such depressions are progressive, increasing in severity with increasing duration of infection and deterioration in clinical condition. Although intake has been reduced with as few as 38 flukes (Duwel, Sambeth and Bossaller, 1972), most studies suggest that the threshold is higher. For example, Hawkins and Morris (1978), failed to reveal any effect on appetite in association with burdens of up to 117 flukes derived from a single infection in sheep, but trickle infections produced intake reductions of 15% with fluke burdens ranging from 87 to 233 (Sykes et al., 1980). In another study 200 flukes did not affect food intake at all when animals were maintained on a high protein diet, but with 500 flukes appetite fell progressively from around 6 weeks post-infection (Berry and Dargie, 1976). There appears therefore to be no direct correlation between adult fluke burdens and the degree of appetite depression.

In cattle, the effect on intake has been examined with less detail and accuracy, but the one detailed study indicated minimal reductions with a burden of 54 flukes, and reductions of 5-10% in association with 140 flukes from around patency (Hope Cawdery, Strickland, Conway and Crowe, 1977).

In spite of its obvious importance, it is not clear why trematode infections depress appetite (Symons, 1985). In the case of *Fasciola* infections, however, appetite is not significantly affected during fluke migration except in severe infections, suggesting that liver damage *per se* is generally unimportant. There is nevertheless a good correlation between the onset and severity of feed intake depression and the development of anaemia and hypoalbuminaemia in association with adult fluke infections. This suggests that the extent of deteriorations in haematological and other blood indices somehow also determine the level of feed intake (Berry and Dargie, 1976). In this context, anaemia *per se* caused by phlebotomy has been found to reduce feed intake and growth in sheep (Alexander and Kiesel, 1965).

6.5.2 *Feed conversion efficiency*

Sykes et al. (1980) discovered that there is a reduction in utilization of metabolisable energy together with a reduction in protein deposition and bone mineral deposition. They considered that there is no specific effect on digestibility. In an infected animal, to maintain the synthesis of the normal amount of plasma protein and haemoglobin, the limited reserves of labile proteins are mobilized and then expendable tissue proteins are utilised with a consequent loss of weight (Reid et al., 1973).

CHAPTER SEVEN

MATERIALS AND METHODS

7.1 Infective Material

7.1.1 *Source of metacercariae*

F. hepatica metacercariae used to infect the sheep in experiment 1 were purchased from the Compton Paddock Laboratories, Newbury, Berks, England. The metacercariae had been shed during the last week of February 1990. *F. gigantica* metacercariae for the same experiment were supplied by Mr H R Urquhart of the CTVM, Edinburgh. These metacercariae were obtained from naturally infected *L. natalensis* in Karatina, Kenya during mid March 1990.

Metacercariae of *F. gigantica* used in the experiments 2, 3 and 5 were obtained from *L. auricularia* race *rufescens* snails reared and infected in the laboratory at the Pakhribas Agricultural Centre, Dhankuta, Nepal. The metacercariae were shed between 25th June and 18th August 1991. *F. gigantica* metacercariae used to infect buffalo calves of experiment 4 were obtained from naturally infected *L. auricularia* race *rufescens* in eastern Nepal between 10th and 20th December 1990.

7.1.2 *Production of metacercariae in the laboratory*

The techniques used were those described by Hammond (1970) with slight modifications.

7.1.2.1 Management of snails in the laboratory

The colonies of *L. auricularia* race *rufescens* were already available at PAC, Dhankuta. The snails were obtained from a local natural habitat. The breeding colonies were maintained in silicone sealed glass aquaria (600 x 300 x 375 cm) containing about 10 litres of filtered spring water and provided with forced aeration. The temperature in the aquaria was maintained at about 25°C using thermostatic heaters. The fluorescent lighting above each aquarium was left on permanently which helped the growth of algae in the aquarium to feed the snails. However, fish food flakes (Tropical Fish Food, Vetra Betta, Singapore) were given as a supplementary food every alternate day. The water was syphoned off together with snail faeces and detritus fortnightly, then replaced with fresh spring water.

7.1.2.2 Obtaining miracidia

F. gigantica eggs were collected from the gall bladder of an infected buffalo at PAC, Dhankuta. The bile was washed through a 150 μm aperture sieve (Endecotts Ltd. London) and into a 38 μm aperture sieve in which the eggs were trapped. The eggs were transferred into an amber bottle using a fine jet of water, and washed by sedimentation in distilled water. The distilled water was changed daily for seven days after which the bottle was wrapped in aluminium foil to make it light tight before being kept in the incubator at 24°C. Miracidia could be induced to hatch after 15 days of incubation. Either a drop in temperature (4°C) or exposure to light triggers the hatching mechanism. The required number of miracidia were picked up with a fine-drawn pasteur pipette under the stereomicroscope. Miracidia less than 3 hours old were always used to infect snails.

7.1.2.3 Infecting snails

Snails, 6-9 mm long, were placed individually into polystyrene bijou bottles, each of which contained a little distilled water containing the infective dose of miracidia (approximately 1 miracidium/mm of snail length). The bottles were then loosely capped and left overnight at room temperature for infection to occur. Each batch of 30-40 infected snails was maintained in separate aquarium under the conditions already described (Section 7.1.9.1.). By 5-6 weeks post-infection the rediae and cercariae were clearly seen inside the infected snails when they were turned onto their backs and examined with a stereomicroscope in the area immediately behind the shell opening.

7.1.2.4 Harvesting metacercariae

Shedding of cercariae was induced 6 weeks after infection by placing the snails with mature infections into a small polythene bag (120 x 75 mm) half filled with distilled water at 4°C. The polythene bag was supported in a beaker and a rubber band was used to close the top. The beaker was left at room temperature under the light from a table lamp fitted with a 100 watt bulb. Shedding commenced as the water warmed up to room temperature. The snails were removed after 5 to 6 hours and returned to the aquaria. The bag containing the shed cercariae was then re-closed and left at room temperature overnight.

The following morning, water in the bag was poured away intó a beaker and any debris was washed off the polythene bag into the same beaker. The portion of the bag containing encysted metacercariae was placed in a screw capped glass universal bottle, one fourth filled with distilled water. The bottle was sealed and labelled, and then stored at 4°C until further use. All the contaminated materials were autoclaved and the working surface was swabbed with methyl alcohol to kill any metacercariae which may have been present.

7.1.3 *Harvesting metacercariae from naturally infected snails*

L. auricularia race *rufescens* snails were collected from the natural habitats located between an elevation of 1,200 and 1,350 m around PAC, Dhankuta. The prevalence of fasicolosis in the animals frequenting these habitats has been reported to be very high but no *Fasciola* spp. other than *F. gigantica* has been found in this area (Morel and Mahato, 1987).

About 50 snails were placed in each polythene bag (250 x 140 mm) filled with cold spring water (4°C). The bag was closed with a rubber band and placed in a tray under direct sunlight. Within 4 to 5 hours, cercariae were shed if the bag contained any snail with mature infections. The cercariae were allowed to encyst on the inner surface of the bag overnight.

The grey-white cysts were considered to be those of *F. gigantica*. Further identification of the cysts was done indirectly by examining morphology of the rediae and cercariae obtained on dissection of the snails from the bags containing cysts, using the criteria described by Frandsen and Christensen (1984) and Ollerenshaw and Graham (1986). The metacercariae were stored at 4°C as described earlier. Metacercariae which could not be identified were discarded.

7.2 Experimental Animals and Design

7.2.1 *Experiment 1: Comparative pathogenicity of F. gigantica and F. hepatica in sheep*

Twelve sheep, 2 male and 4 female Blackface, and 4 male and 2 female Suffolk cross, all approximately 18 months old and raised under fluke-free conditions, were obtained from the Firth Mains Farm of Moredun Research Institute, Edinburgh. The sheep were dosed orally with oxfendazole (Systamex, Wellcome Foundation Ltd., London) at a rate of 5 mg/kg body weight to eliminate nematode worms, and thereafter housed in concrete pens provided with wood shavings as bedding. The sheep were fed a daily ration of 300 g cubes (dry matter 88%; protein 18%, oil 2.5%, fibre 10% and ash 8% of dry matter) per head. Chopped hay and drinking water were available *ad libitum*.

Table 7.1. Experimental details of the study on comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep.

Group	Sub-group	Animal No.	Breed	Sex	Body weight (kg)	Cysts given (No)	Infective dose (cysts/kg)	Mean infective dose (cysts/kg)
A	A_1	24	Suffolk X	F	44.5	150	3.4	3.1
	(lower dose)	28	Suffolk X	M	54.0	150	2.8	
F. hepatica	A_2	26	Blackface	F	29.0	350	12.6	11.3
infected	(higher dose)	30	Blackface	M	35.5	350	9.9	
B	B_1	23	Suffolk X	F	45.0	150	3.3	3.2
	(lower dose)	27	Suffolk X	M	48.5	150	3.1	
F. gigantica	B_2	25	Blackface	F	25.5	350	13.7	11.4
infected	(higher dose)	29	Blackface	M	39.0	350	9.0	
C	C_1	21	Suffolk X	F	48.0	0	0.0	0.0
	(controls)	22	Suffolk X	M	53.0	0	0.0	
Uninfected	C_2	31	Blackface	M	43.0	0	0.0	0.0
	(controls)	32	Blackface	F	26.0	0	0.0	

The sheep were divided into three groups of four animals so that each group was represented by an equal number of animals of the same breed and sex. Also, the mean liveweight for each group was more or less equal. The animals of each group were further sub-divided into two pairs. The two groups to be infected were selected at random and then designated as A and B. One pair from group A was infected with 150 and another with 350 metacercariae of *F. hepatica*. Similarly, the first pair from group B was infected with 150 and the second pair with 350 metacercariae of *F. gigantica* . Both pairs of sheep in group C constituted the uninfected control. The experimental design and the details of each animal are shown in Table 7.1.

7.2.2 *Experiment 2: Experimental F. gigantica infection in Nepalese Baruwal sheep*

For this experiment, nine female Baruwal sheep aged between 11 and 14 months were purchased locally from a migratory flock. The faecal samples of the sheep were negative for *Fasciola* spp. eggs but heavily positive for gastrointestinal nematode eggs. Sera from each animal were examined for *Fasciola* antibodies by an agar gel diffusion (Section 7.4.5); all the sheep were found negative.

All the sheep were treated parenterally against ecto- and endo-parasites with ivermectin (Ivomec, MSD-AGVET, U.K.) and praziquantel (Droncit, Bayer U.K. Limited). They were immunized against pasteurellosis, lamb dysentery, struck, pulpy kidney, tetanus, braxy, black leg and black disease using Heptavac-P (Hoechst U. K. Limited). Regular treatment against gastrointestinal nematodes was carried out at two month intervals for the whole period of experiment. The sheep were housed in concrete pens at nights but they were tethered to the feeding racks on gravelled ground under a sun shed during the day. They received a commercial concentrate feed at a rate of approximately 300 g per sheep every morning followed by assorted tree fodder *ad libitum*. Drinking water was offered twice a day.

The sheep were randomly divided into three groups of three animals. Each sheep in the groups A and B was infected with 285 and 125 *F. gigantica* metacercariae so that the mean infective doses were 9.9 and 4.3 cysts per kg body weight respectively (Table 7.2). The sheep in group C were kept as uninfected controls.

Table 7.2. Experimental design of the study on *F. gigantica* infection in Nepalese Baruwal sheep.

Group	Animal No.	Body wt. at infection (kg)	Mean body wt. (kg)	*F. gigantica* cysts given (No.)	Infective dose (cysts/kg)	Mean infective dose (cysts/kg)
A	11 12 13	30 28 28	28.7	285 285 285	9.5 10.2 10.2	9.9
B	14 15 16	32 29 26	29.0	125 125 125	3.9 4.3 4.8	4.3
C	17 18 19	32 24 24	26.7	0 0 0	0 0 0	0

7.2.3 *Experiment 3: Experimental F. gigantica infection of Nepalese hill goats*

Eighteen local goats, all negative for *Fasciola* spp. infection on faecal examination and the agar gel diffusion test were used in this study. The goats were castrated males between 15 and 18 months old. Anthelmintic dosing, vaccination, housing and feeding of the goats were similar to those as described for sheep in Section 7.2.2.

The goats were divided into three groups of six animals so that the mean live weights of the groups were statistically equal (Table 7.3). The groups to be infected were selected at random, designated as A and B and then infected with 185 and 80 cysts of *F. gigantica* respectively. The goats in the third group (group C) were retained as uninfected controls.

Table 7.3. Experimental design of the study on *F. gigantica* infection in Nepalese hill goats.

Group	Animal No.	Body wt. at infection (kg)	Mean body wt. (kg)	*F. gigantica* cysts given (No.)	Infective dose (cysts/kg)	Mean infective dose (cysts/kg)
A	31 32 33 34 35 36	21 16 18 18 21 18	18.7	185 185 185 185 185 185	8.8 11.6 10.3 10.3 8.8 10.3	9.9
B	41 42 43 44 45 46	21 21 17 18 21 20	18.8	80 80 80 80 80 80	3.8 5.0 4.7 4.4 3.8 4.0	4.3
C	51 52 53 54 55 56	20 17 17 16 22 18	18.3	0 0 0 0 0 0	0 0 0 0 0 0	0

7.2.4 *Experiment 4: Experimental infection of Nepalese hill buffaloes with high dose of F. gigantica metacercariae*

Sixteen indigenous buffalo calves obtained locally from high altitude (above 1,700 m) farms were used. The calves were 12 to 18 months of age. They were examined clinically and faecal examinations were carried out several times to ensure that the animals were free from fasciolosis and any clinically detectable disease.

All the calves were treated with ivermectin (Ivomec, MSD-AGVET, U.K.) to eliminate gastrointestinal nematodes. Throughout the period of observation, the calves were kept under the management, housing and feeding system similar to those as described for sheep (Section 7.2.2), except that each calf received appróximately 1 kg commercial concentrate feed daily.

The buffalo calves were paired on the basis of their sex and liveweight and then divided into two groups so that each group of eight animals consisted of one animal from each pair. The group to be infected (group A) was randomly selected and each calf in this group was given 1,500 metacercariae. The calves in the other group (group B) consisted the uninfected controls. Details of the individual animal and the experimental plan are shown in Table 7.4.

Table 7.4. Experimental details of the study on high level *F. gigantica* infection in buffaloes

Group	Animal No.	Sex	Age (month)	Body weight (kg)	Mean body wt. (kg)	*F. gigantica* cysts given (No.)	Infective dose (cysts/kg)	Mean infective dose (cysts/kg)
A	81	M	16	97	106	1500	15.5	14.2
	83	F	12	100		1500	15.0	
	85	F	14	82		1500	18.3	
	87	M	18	120		1500	12.5	
	89	M	18	105		1500	14.3	
	92	M	14	97		1500	15.5	
	94	M	18	117		1500	12.8	
	96	M	18	129		1500	11.6	
B	82	M	16	113	105	0	0.0	0.0
	84	F	14	97		0	0.0	
	86	F	15	70		0	0.0	
	88	M	18	128		0	0.0	
	90	M	18	110		0	0.0	
	91	M	15	93		0	0.0	
	93	M	16	93		0	0.0	
	95	M	18	137		0	0.0	

7.2.5 *Experiment 5: Experimental infection of Nepalese hill buffaloes with low dose of F. gigantica metacercariae*

Sixteen indigenous buffalo calves, negative for *Fasciola* infection on faecal examination and agar gel diffusion test were purchased locally for this experiment. They were approximately 11 to 16 months old.

Anthelmintic dosing, housing and feeding systems were that which have already been described for buffaloes in the Section 7.2.4. Additionally, the calves used in this experiment were vaccinated with Heptavac-P (Hoechst, U.K.) against the bacterial diseases mentioned in the Section 7.2.2.

The grouping procedure and the experimental design were similar to that in experiment 4 except that the dose of metacercariae given to each animal in the infected group (group A) was decreased to 400. The details of the experiment are presented in Table 7.5

Table 7.5. Experimental details of the study on low level *F. gigantica* infection in buffaloes

Group	Animal No.	Sex	Age (month)	Body weight (kg)	Mean body wt. (kg)	*F. gigantica* cysts given (No.)	Infective dose (cysts/kg)	Mean infective dose (cysts/kg)
A	61	M	12	87	92	400	4.6	4.4
	62	M	14	105		400	3.8	
	63	F	12	84		400	4.8	
	64	F	11	69		400	5.8	
	65	M	15	118		400	3.4	
	66	M	13	90		400	4.4	
	67	M	12	84		400	4.8	
	68	M	14	99		400	4.0	
B	71	M	12	86	92	0	0.0	0.0
	72	M	12	99		0	0.0	
	73	F	13	95		0	0.0	
	74	F	12	61		0	0.0	
	75	M	14	103		0	0.0	
	76	M	14	104		0	0.0	
	77	M	12	82		0	0.0	
	78	M	16	106		0	0.0	

7.3 Infection of Animals

The polythene bag containing metacercariae was opened with scissors and cut into manageable pieces. The metacercariae were examined for viability under a stereomicroscope at 50x. The metacercariae having a characteristic granular appearance were considered as viable (Hammond, 1970).

The required number of metacercariae were counted and then scraped and washed off the polythene in a plastic petri dish using a microscope glass slide as scraper. The water containing metacercariae was poured on to a sheet of Whatman No. 4 filter paper in a glass funnel ensuring that no metacercariae had been left in the petri dish. After the paper had drained, it was folded, with the retained metacercariae inside, and administered orally to the animals using a balling gun.

7.4 Monitoring of Animal Experiments

7.4.1 *Measurements of weight gain*

The animals were weighed at weekly intervals. A weighing crush (Weigh-bridge, Leslie P. Morris Ltd, Salop) was used to weigh the sheep in the experiment at CTVM. A 200 kg spring balance (Salter India Ltd.) for buffaloes and a 50 kg spring balance (Salter India Ltd.) for sheep and goats were used at PAC. All weighing were carried out from 0800-1000 hours before feed was provided to the animals.

7.4.2 *Sampling and storage of the samples*

Faecal samples were collected directly from the rectum and examined the same day for helminth eggs. The blood samples for haematological studies were taken from the jugular vein into vacutainers containing disodium or dipotassium ethylenediamine tetra-acetic acid (EDTA) as an anticoagulant and examined the same day. The blood samples for biochemistry and serology were obtained from the jugular vein into plain vacutainers (Becton, Dickinson & Co Ltd, U K) and allowed to clot in the incubator at 37°C for 1 hour, then kept at 4-6°C for 1 hour for the clot to retract. The serum was separated by centrifugation at 2,500 g for 20 minutes, dispensed into 1 ml aliquots and stored in labelled Eppendorf tubes frozen at -20°C until required. All sampling was carried out at weekly intervals between 0830 and 0930 hours.

7.4.3 *Haematological techniques*

7.4.3.1 Total leucocyte and erythrocyte counts

These were determined by the method described in the Reference Manual for the Coulter Counter Model ZM (Coulter Electronics, 1985), using an electronic cell counter (Coulter Counter Model ZM, Coulter Electronics Ltd, Luton). The dilution used for the total leucocyte count was 1:500 whereas for the total erythrocyte count a dilution of 1:25,000 was used.

7.4.3.2 Eosinophil counts

These were done by a modification of the method of Dacie and Lewis (1963) using a modified Fuschs Rosenthal Chamber (Weber Scientific International Ltd, Lancing, England), 0.2 ml of blood being added to 0.8 ml of Discombe's fluid (5 ml eosin 1%, 5 ml acetone and 90 ml distilled water). Two of the chambers were always filled and all the eosinophils counted.

7.4.3.3 Packed cell volumes

This was determined using a Hawksley micro-haematocrit centrifuge and reader (Hawksley and Sons Ltd, Lancing, England). The samples were centrifuged at 12,000 g for 7 minutes and the volumes were expressed in litre per litre.

7.4.3.4 Haemoglobin estimation

Haemoglobin converted to cyanmethaemoglobin with Zapoglobin (Coulter Electronic Ltd, Luton) was measured in a Haemoglobinometer (Coulter Electronic Ltd, Luton). The haemoglobin concentrations were expressed as grams per litre.

7.4.4 *Biochemical techniques*

7.4.4.1 Total serum protein

Total serum protein was determined by the Biuret method described in Total Protein Procedure No. 541 (Sigma Diagnostics, 1989) using the Total Protein Reagent (541-2, Sigma) and Protein Standard (540-10, Sigma). The procedure is based on the principle that the copper ions in alkaline biuret reagent, react with the peptide bonds of serum proteins to form a purple colour with an absorbance maximum at 540 nm. The intensity of colour is proportional to the total protein concentration.

A spectrophotometer (PU 8600 UV/VIS, Pye Unicam Ltd, Cambridge) was used to measure the optical density. The reliability of test results were monitored by routine use of control sera, Accutrol™ Normal (A-2034, Sigma) and Accutrol™ Abnormal (A-3034, Sigma).

7.4.4.2 Serum albumin

Serum albumin was estimated by a method described in Albumin Procedure No. 631 (Sigma Diagnostics, 1988) using Sigma Albumin Reagent (631-M, Sigma) and Protein Standard (540-10, Sigma). The bromcresol green in the Albumin Reagent binds to albumin to produce a blue green colour with an absorbance maximum at 628 nm. The intensity of the colour produced is directly proportional to albumin concentration in the sample.

The absorbance was measured using the Pye Unicam PU 8600 UV/ VIS spectrophotometer. The results were expressed in gram per litre. The Accutrol™ preparations (A-2034 and A-3034, Sigma) were used for quality control purposes.

7.4.4.3 Serum globulin

Serum globulin values were calculated as the differences between the total serum total protein and serum albumin. The values were expressed in gram per litre.

7.4.4.4 Serum glutamate dehydrogenase (GLDH)

The activity of GLDH (EC 1.4.1.3) in the serum was measured, without determination of non-specific creep reaction, using a kit, Test-Combination GLDH activated (124320, Boehringer Mannheim). The test is based on the following principal:

$$\alpha\text{-oxoglutarate} + NADH + NH_4^+ \xrightleftharpoons{GLDH} \text{glutamate} + NAD^+ + H_2O$$

Spectrophotometric readings were made at 25°C and 340 nm. Precinorm U and Precipath U (Boehringer Mannheim calibrated standards) were used in the quality assurance checks. GLDH activities in the samples were expressed as Unit per litre which were calculated using the manufacturer's instruction.

7.4.4.5 Serum gamma glutamyl transpeptidase (γ-GT)

The activity of γ-GT (EC 2.3.2.2) in the serum was assayed using a kit, τ-GT MPR 1 (1087584, Boehringer Mannheim) which is based on the following principal:

$$\text{L-}\gamma\text{-glutamul-3-carboxy-4-nitroanilide + glycylglycine} \xrightarrow{\gamma\text{-GT}} \text{L-}\gamma\text{-glutamyl glycylglycine + 5-amino-2-nitrobenzoate}$$

Spectrophotometric readings were taken at 25°C and 405 nm. The calibrated standards, Precinorm U and Precipath U were used in the quality assurance checks made on the assays performed. The activities of γ-GT in the samples were expressed as Unit per litre.

7.4.4.6 Serum calcium

Serum calcium was determined using a kit (587-M, Sigma) as per the procedure described in Calcium Procedure No. 587 (Sigma Diagnostics, 1990). The procedure for this kit is based on the principal that the reaction of calcium with 0-cresolphthalein complexone produces a red complex at pH 10-12. The intensity of the colour, measured at 575 nm, is directly proportional to calcium concentration in the sample. The Sigma Accutrol^{TM} preparations were used for the quality control purposes.

7.4.4.7 Serum potassium and serum sodium

Serum potassium and serum sodium were determined flame-photometrically in accordance with Janway Application Advice No.3 (Janway, 1988) using a flame photometer (PFP7, Janway). The photometer was set up with either a K or a Na filter depending on the mineral determination. The instrument was adjusted to obtain 100 on the appropriate standard (1.0 mg K or 1.0 mg Na per 100 ml) and zero reading on the blank (deionised distilled water). The haemolysis-free serum sample prepared to a 1:50 dilution in deionised distilled water was sprayed and the reading was noted. The concentration equivalent to this reading was read off from the appropriate calibration curve which was prepared previously using a range of standard solutions at the concentration of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/100 ml. The concentration of the mineral in the serum (mg/100 ml) was calculated by multiplying the dilution factor of 50 and then converted to mM/l.

7.4.4.8 Serum magnesium

This was determined colorimetrically by the method described in Magnesium Procedure No. 595 (Sigma Diagnostics, 1987) using a kit (595-A, Sigma). This technique is based on the reaction that an unmetallized form of dye (calmagite) in the presence of magnesium forms a pink magnesium-calmagite complex which can be measured at 520 nm. The Sigma Accutrol^{TM} preparations were used for the quality control purposes.

7.4.4.9 Serum inorganic phosphorus

Serum inorganic phosphorus was estimated by the method described in Phosphorus, inorganic, Procedure No. 670 (Sigma Diagnostics, 1985) using a kit (670-C, Sigma). The serum was treated with trichloroacetic acid to remove protein and lipid phosphorus. The supernatant fluid was reacted with ammonium molybdate in an acid solution to form phosphomolybdate. A mixture of sodium bisulfite, sodium sulfite and 1-amino-2-naphthol-4-sulfonic acid (Fiske & SubbaRow reducer) was added to reduce the phosphomolybdate to form a phosphomolybdenum blue complex. The intensity of the colour which is proportional to the phosphorus concentration was measured at 660 nm. The Sigma Accutrol^{TM} preparations were used for the quality control purposes.

7.4.4.10 Serum copper

Serum copper was determined by a standard technique. Copper in blood is bound to ceruloplasmin and albumin. This is split off with hydrochloric acid and

Cu^{++} is reduced with pyro-sulphite to Cu^{+}. A coloured complex is formed with bathocuproin disulphonate, the colour intensity of which is proportional to the copper concentration.

One ml protein precipitant (10% trichloroacetic acid in 0.5 N hydrochloric acid) was dispensed into three 10 ml tapered centrifuge tubes. One ml of each test serum, standard solution (2.54 μg Cu per ml) and blank (deionised distilled water) was added to tube 1, 2 and 3 respectively. After thorough mixing, the solution was allowed to stand for 20 minutes at room temperature and then centrifuged at 2,500 g for 10 minutes. One ml of clear supernatant was transferred to a clean test tube and to each tube, 0.5 ml colour reagent (125 g anhydrous sodium acetate, 47.8 g sodium bisulphite and 425 mg bathocuproin disulphonate in 1 litre deionised distilled water) was added. The solution was well mixed and then the optical density of the unknown and the standard against the reagent blank was read at 450 nm. All estimations were made in duplicate and the mean optical densities were used to calculate the copper concentration of the sera.

7.4.5 *Agar gel diffusion assay*

This 'traditional' serological technique, agar gel diffusion (AGD) test was used for the monitoring of infected and uninfected animals.

The parasite extract for use as 'antigen' was prepared from adult *F. gigantica* collected post mortem from a buffalo as described by Lehner and Sewell (1980). After removal from the bile ducts, the flukes were held at 37°C for 1-2 hours, washed in several changes of chilled 1M phosphate buffered saline (PBS), and then 20 flukes were homogenised in a suspension with 10 ml 1 M PBS (pH 7.2). The homogenate, after leaving overnight at 4°C, was centrifuged at 5,000 g at 4°C for 30 min. The supernatant, which was used as 'antigen', was stored frozen at -20°C until further use.

The AGD procedure described by Swarup, Pachauri and Sharma (1987) was followed. Basic medium used for the gel was 1% Noble agar (Difco) in 0.85% (w/v) saline containing 0.2% sodium azide. Agar was poured on to 15 cm x 10 cm glass plates at a thickness of 2 mm and the wells of 4 mm diameter 3 mm distance apart were punched. The surrounding wells were filled with test sera (undiluted), while central wells were used for the antigen. Diffusion was allowed for 24 hours at 37°C in a humid chamber and precipitation lines were stained with amido black.

7.4.6 *Helminthological techniques*

7.4.6.1 Faecal egg counts

The method used was the differential centrifugal flotation technique (Sewell and Hammond, 1972) (described in Section 3.6.1). After centrifugation of the sample in saturated salt solution, the centrifuge tube was carefully topped up with saturated salt solution until a slight convex meniscus was formed. A cover slip was placed over the meniscus, left to stand for about 5 minutes, and then lifted off vertically, placed on a microscope slide and examined for the presence of gastro-intestinal nematode eggs. The sediment was further processed for the detection of *Fasciola* eggs as previously described (Section 3.6.1).

7.4.6.2 Recovery of flukes from infected livers

The method used for recovery, counting and measuring the flukes are described in Sections 3.6.2 and 3.6.3.

7.4.7 *Pathological techniques*

7.4.7.1 Post mortem procedures

Post mortem examinations were carried out on all animals as soon as possible after they died or had been humanely killed. A careful examination was made of all internal organs for abnormalities, with particular attention to their colour and appearance. The lungs were examined for ectopic flukes and, if these were present, selected areas were removed for histopathological examination. The same procedure was followed for lesions found in other organs, except the liver which was removed and dealt with separately. The rumen and reticulum were examined for paramphistomes, and the pancreas of all the animals for *Eurytrema* spp. The abomasum and the small and large intestines were examined separately for helminths. In all cases where there was abscess formation, or other evidence of bacterial infection, specimens were submitted for examination.

7.4.7.2 Histopathology

Samples of liver and other tissues were fixed in 10% buffered formol-saline. Histological slides stained with haematoxylin and eosin (H&E), Maritus scarlet blue (MSB), carbolchromotrope (CC) and Prussian Blue Reaction (PBR) were prepared at the Veterinary Field Station, Department of Pathology, Royal (Dick) School of Veterinary Studies.

7.5 Data Handling and Statistical Analysis

Collations and initial analyses of the data were done using the computer and softwares mentioned in Section 3.7. In addition to the statistical packages mentioned in Section 3.7, GraphPAD INSTAT, version 1.4 (GraphPAD Software) was also used for the statistical analyses.

The differences in liveweight gains between the groups of animals were carried out by comparing the slopes of the regression lines using one way analysis of variance (ANOVA). The comparison of the haematological and biochemical values between the groups at different weeks were carried out by the non-parametric Mann-Whitney test; however, in experiment 3 in which there were 3 groups of animals, the Kruskal-Wallis one-way analysis of variance was used.

The correlations between the fluke burden and the liveweight gains, and haematological and biochemical values were assessed by calculating the Pearson's Correlation Coefficients and their probabilities. In all analyses, the significance level was taken as $P < 0.05$. Further details of the data analysis are described with the relevant results.

CHAPTER EIGHT

RESULTS

8.1 Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

8.1.1 *Clinical data*

All 8 infected sheep were clinically normal until 11 WPI. Thereafter, sheep 23, 27 (given 150 *F. gigantica* metacercariae) and 25 (given 350 *F. gigantica* metacercariae) had loss of appetite, abdominal distension and showed signs of anaemia. The condition of these sheep deteriorated progressively. Sheep 23 died 14 WPI whereas sheep 25 was slaughtered 15 WPI while in extremis. A day before death, both sheep were restless, unable to stand (Figure 8.1.1) and had dyspnoea and subnormal body temperature. Except for sheep 26 (given 350 *F. hepatica* metacercariae) which had loss of appetite around 10 WPI, none of the remaining 4 infected or any uninfected sheep showed clinical signs during the experimental period.

8.1.2 *Liveweight data*

The changes in liveweight of the sheep are shown Figure 8.1.2 and Appendix Table 8.1.1. Depression in liveweight of all the sheep seen at 5 WPI was due to shearing them the day before weighing. The infected sheep lost weight between 8 and 11 WPI and grew at a slower rate thereafter. However, the infected sheep gained weight over the experimental period, although less than the uninfected controls, except for sheep 27 (given 150 *F. gigantica* metacercariae) which lost 2.5 kg.

8.1.3 *Parasitological data*

The prepatent period for the *F. gigantica* infections ranged from 90 to 101 days while that for *F. hepatica* ranged from 68 to 84 days (Table 8.1.1). Soon after patency, a peak in the faecal egg counts (EPG) was observed in *F. hepatica* infected

Figure 8.1.1. Sheep 25 (right) with a burden of 181 *F. gigantica*, a day before slaughter at 15 WPI and sheep 26 (left) with a burden of 247 *F. hepatica*.

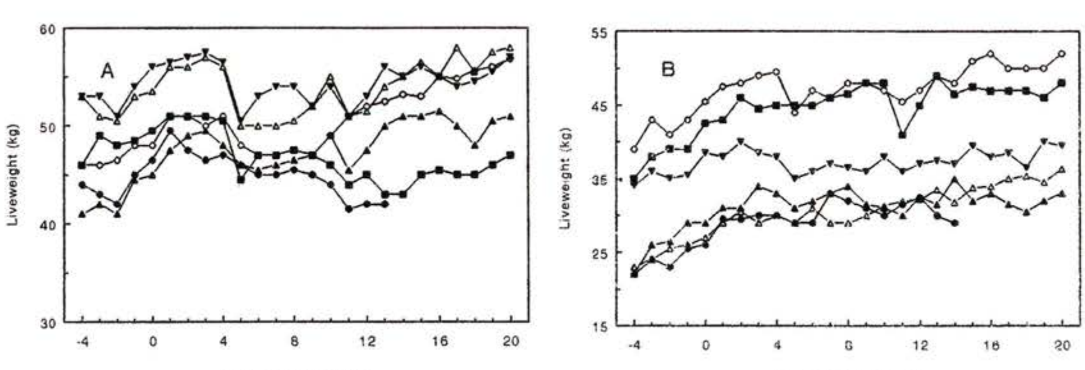


Figure 8.1.2. Liveweights of sheep in experiment 1. (A) sheep 23 (●) and 27 (■) infected with 150 *F. gigantica* metacercariae, sheep 24 (▲) and 28 (▼) infected with 150 *F. hepatica* metacercariae and uninfected control sheep 21 (O) and 22 (△). (B) sheep 25 (●) and 29 (■) infected with 350 *F. gigantica* metacercariae, sheep 26 (▲) and 30 (▼) infected with 350 *F. hepatica* metacercariae and uninfected control sheep 31 (O) and 32 (△).

Table 8.1.1. Parasitological details of the sheep infected with *F. gigantica* and *F. hepatica* in experiment 1.

Sheep	Treatments (number and spp. of metacercariae given)	Duration of infection (weeks)	Prepatent period (days)	Fluke recovery		Mean (± SD) fluke size (mm)	
				No.	%	Length	Width
23	150 *F. gigantica*	14	93	80	53.3	25.2 (± 3.7)	4.0 (± 0.9)
27	150 *F. gigantica*	20½	92	83	55.3	41.0 (± 2.3)	10.6 (± 1.9)
24	150 *F. hepatica*	20½	73	77	51.3	31.0 (± 3.8)	13.4 (± 1.4)
28	150 *F. hepatica*	20½	68	95	63.3	32.9 (± 2.3)	13.8 (± 1.2)
25	350 *F. gigantica*	15	101	181	51.7	18.8 (± 3.3)	2.7 (± 0.7)
29	350 *F. gigantica*	20½	90	34	9.7	44.9 (± 1.6)	10.7 (± 1.4)
26	350 *F. hepatica*	20½	84	247	70.6	24.4 (± 4.8)	10.9 (± 2.6)
30	350 *F. hepatica*	20½	68	203	58.0	24.8 (± 2.9)	10.2 (± 1.8)

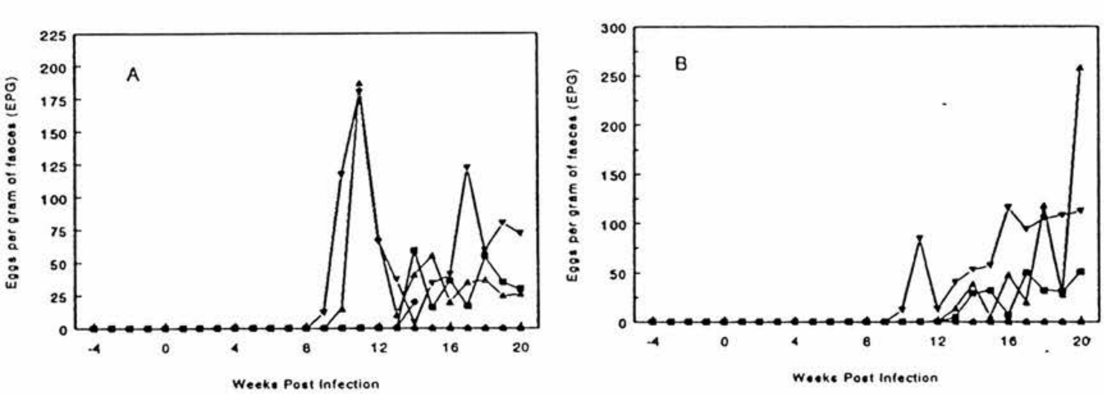


Figure 8.1.3. Faecal egg counts of sheep in experiment 1. (A) sheep 23 (●) and 27 (■) infected with 150 *F. gigantica* metacercariae, sheep 24 (▲) and 28 (▼) infected with 150 *F. hepatica* metacercariae and uninfected control sheep 21 (O) and 22 (△). (B) sheep 25 (●) and 29 (■) infected with 350 *F. gigantica* metacercariae, sheep 26 (▲) and 30 (▼) infected with 350 *F. hepatica* metacercariae and uninfected control sheep 31 (O) and 32 (△).

sheep at 11 WPI (Figure 8.1.3 and Appendix Table 8.1.2). Thereafter, the EPG values rose gradually until the end of the experiment. In general, the faecal egg counts were higher in *F. hepatica* infected sheep than in the *F. gigantica* infected ones.

The recovery of flukes from *F. gigantica* infected sheep ranged from 9.7-55.3 %, the lowest percentage being recovered from sheep 29 which had been given a higher infective dose of 350 metacercariae (Table 8.1.1). The recovery of flukes from *F. hepatica* infected sheep ranged from 51.3-70.6%. In sheep 23 and 25, more than 60% of the flukes were recovered from the liver parenchyma. Some ectopic flukes from sheep 25; 4 from the peritoneal cavity, 3 from the intestinal wall and 1 from the lungs were also recovered. In sheep 27 which was slaughtered at 20½ WPI, about 90% of the flukes were found in the bile ducts while remaining 10% were still in the liver parenchyma. All the flukes recovered from sheep 29 were adults and in the bile ducts. All the *F. hepatica* were adults when recovered from the livers of infected sheep at 20½ WPI, except for sheep 26 in which a few flukes were still migrating in the liver parenchyma. No flukes were recovered from the livers of any uninfected controls.

8.1.4 *Pathological data*

Lesions were widespread in sheep 23 and 25. The carcasses of these sheep were pale; the peritoneal cavity contained large quantities of blood-stained fluid and several blood clots; the small and large intestines were filled with blood and blood stained ingesta (Figure 8.1.4). The livers were enlarged with prominent haemorrhagic tracts and with areas of fibrinous peritonitis on the surface (Figure 8.1.5). The gall bladders were enlarged and filled with clotted blood. The liver of sheep 23 was paler in colour than that of sheep 25 and there was a large blood clot between the liver capsule and the parenchyma. The lungs of sheep 25 showed large areas of congestion (Figure 8.1.6), these areas contained typical haemorrhagic tracts due to a migrating fluke which was found in the subpleura and subpleural oedema was prominent.

The remaining infected sheep had typical lesions of chronic fasciolosis with enlarged livers and bile ducts distended with flukes (Figure 8.1.7).

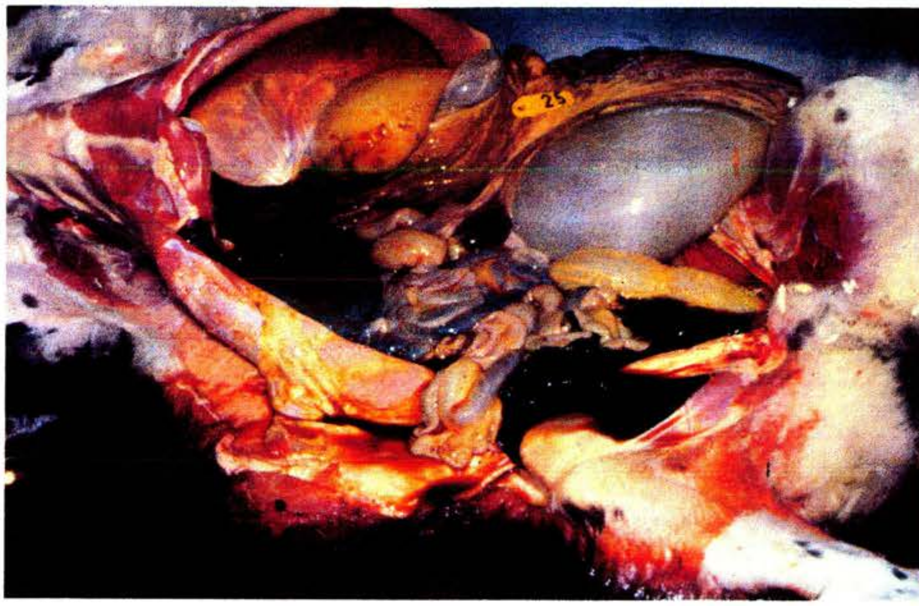


Figure 8.1.4. Carcass of sheep 25 with a burden of 181 *F. gigantica*, slaughtered in extremis at 15 WPI, showing extensive haemorrhages in the abdominal cavity.

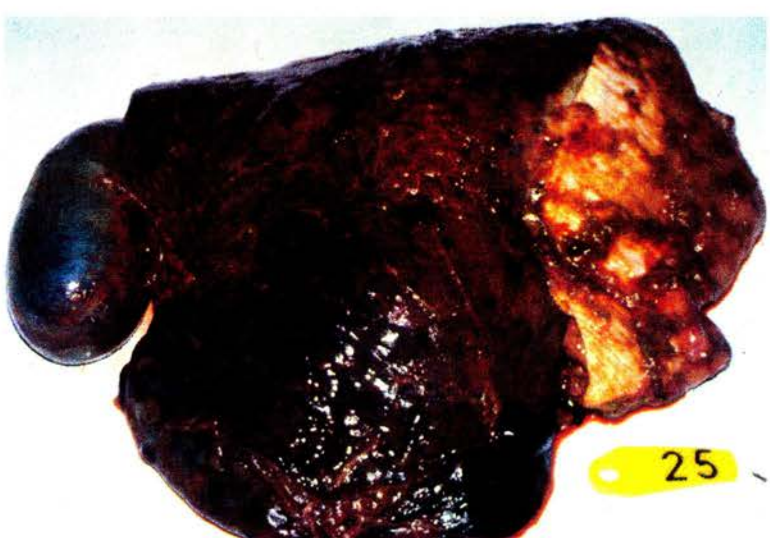


Figure 8.1.5. Liver of sheep 25 with a burden of 181 *F. gigantica*, slaughtered in extremis at 15 WPI, showing lesions on the surface.

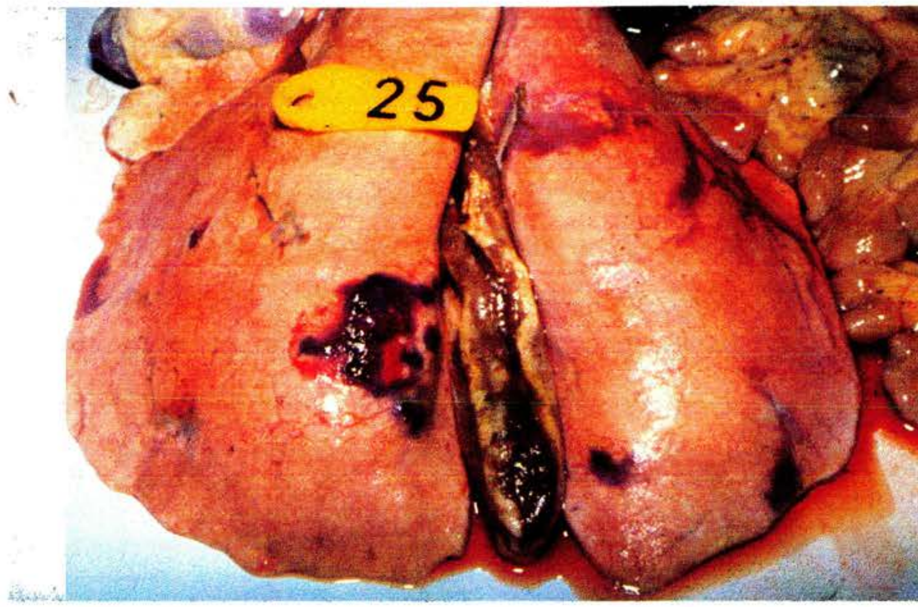


Figure 8.1.6. Lungs of sheep 25 with a burden of 181 *F. gigantica*, slaughtered in extremis at 15 WPI, showing the lesion caused by aberrant migration of a fluke.

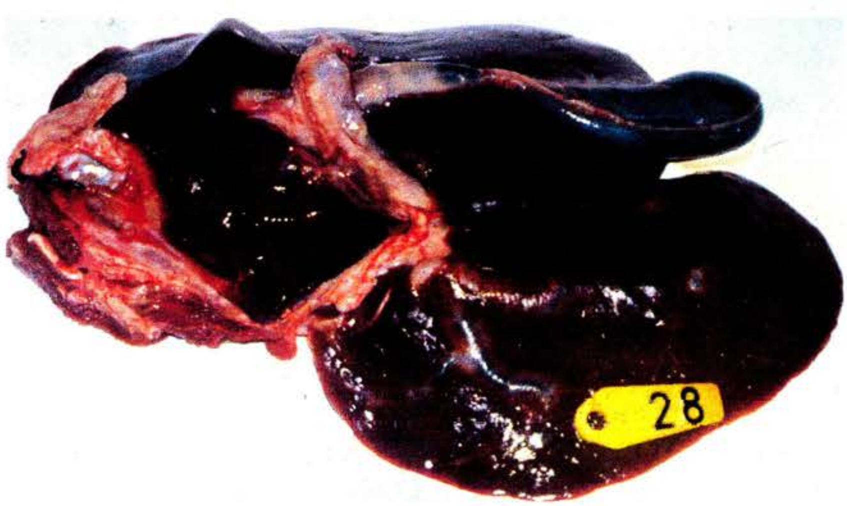


Figure 8.1.7. Livers of sheep 28 with a burden of 95 *F. hepatica*, slaughtered 20½ WPI.

8.1.5 *Haematological data*

All the infected sheep showed a drop in RBC counts after 8 WPI (Figure 8.1.8 and Appendix Table 8.1.3). In sheep 25, the decline became evident as early as 4 WPI. Packed cell volumes (Figure 8.1.9 and Appendix Table 8.1.4) and haemoglobin concentrations (Figure 8.1.10 and Appendix Table 8.1.5) of these sheep also appeared to follow similar pattern of decline. As can be seen from the figures, these declines were more pronounced in *F. gigantica* infected sheep, except for sheep 29 which harboured the lowest number of flukes.

The MCV values indicated a macrocytic anaemia in all the infected sheep (Figure 8.1.11 and Appendix Table 8.1.6). MCH levels in the infected sheep also appeared to rise (Figure 8.1.12 and Appendix Table 8.1.7). However, values for the MCHC showed no changes as a result of either *F. gigantica* or *F. hepatica* infections (Figure 8.1.13 and Appendix Table 8.1.8)

There was a leucocytosis from the 2nd week after infection in most of the sheep, the most pronounced being in sheep 25 with a peak at 5 WPI (Figure 8.1.14 and Appendix Table 8.1.9). In sheep 29, the total WBC counts were at their highest levels 12 weeks after infection.

The eosinophil counts rose by the 2nd week after infection and reached maximum levels shortly after that, except in the case of sheep 26, 28 and 29 where the highest peaks were not reached until 9, 10 and 12 WPI respectively (Figure 8.1.15 and Appendix Table 8.1.10). Following the first peak there was a gradual but irregular fall in values, except in sheep 29 where the eosinophil count fell very rapidly during the following 3 weeks.

8.1.6 *Biochemical data*

The results of the serum GLDH estimations are shown in Appendix Table 8.1.11 and Figure 8.1.16. The values for the uninfected controls (Nos 21, 22, 31 and 32) remained at a uniformly low level, but those of all the infected sheep showed a rise which commenced by 2nd WPI. The maximum level was reached 3-5 WPI, except in the case of sheep 24, 28 and 29 where the highest peaks were not reached

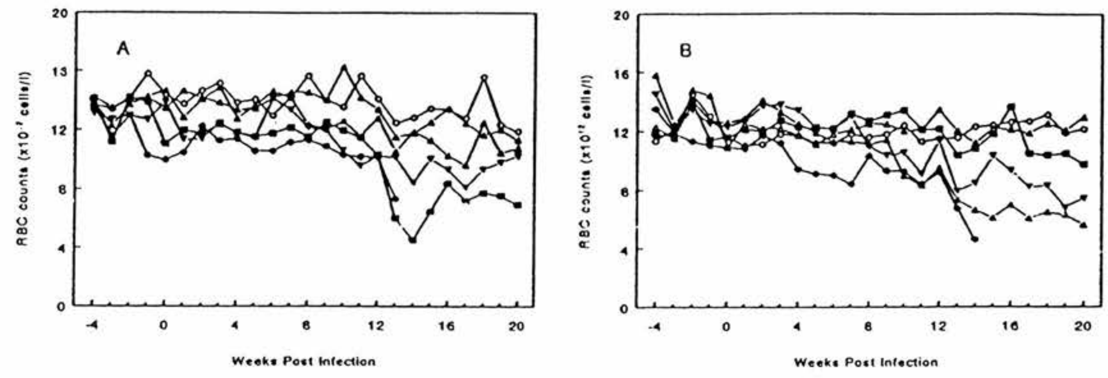


Figure 8.1.8. RBC counts of sheep in experiment 1. (A) sheep 23 (●) and 27 (■) infected with 150 *F. gigantica* metacercariae, sheep 24 (▲) and 28 (▼) infected with 150 *F. hepatica* metacercariae and uninfected control sheep 21 (O) and 22 (△). (B) sheep 25 (●) and 29 (■) infected with 350 *F. gigantica* metacercariae, sheep 26 (▲) and 30 (▼) infected with 350 *F. hepatica* metacercariae and uninfected control sheep 31 (O) and 32 (△).

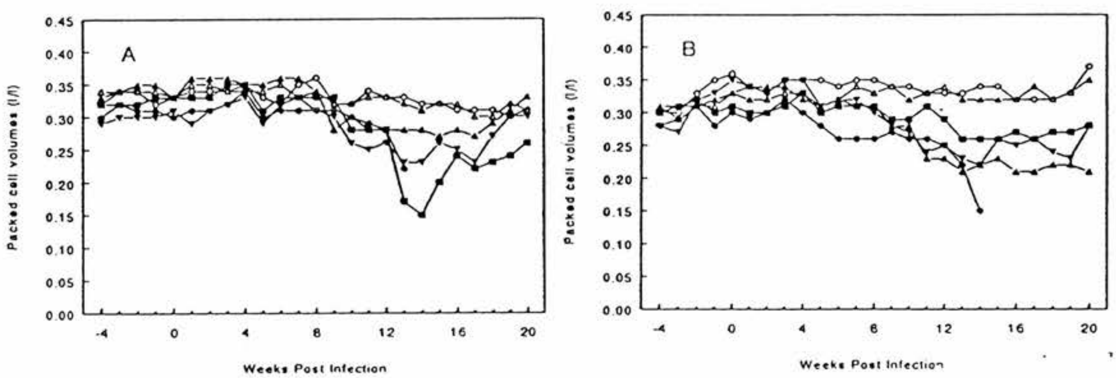


Figure 8.1.9. PCVs of sheep in experiment 1. (A) sheep 23 (●) and 27 (■) infected with 150 *F. gigantica* metacercariae, sheep 24 (▲) and 28 (▼) infected with 150 *F. hepatica* metacercariae and uninfected control sheep 21 (O) and 22 (△). (B) sheep 25 (●) and 29 (■) infected with 350 *F. gigantica* metacercariae, sheep 26 (▲) and 30 (▼) infected with 350 *F. hepatica* metacercariae and uninfected control sheep 31 (O) and 32 (△).

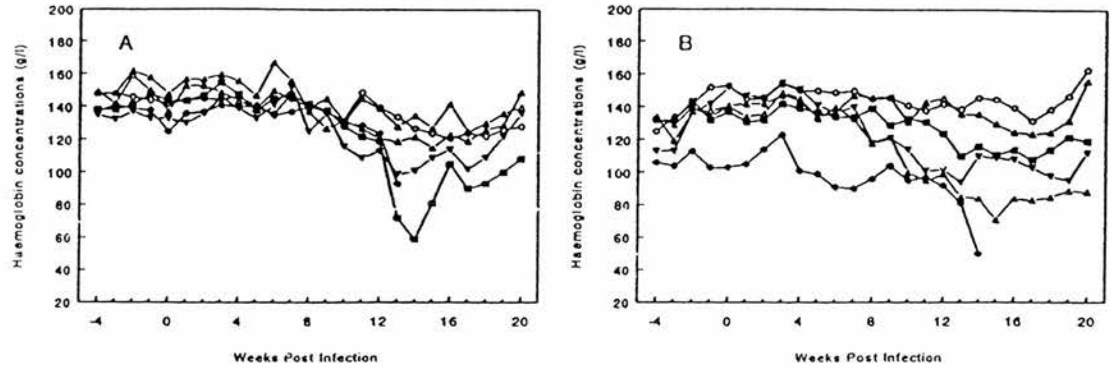


Figure 8.1.10. Haemoglobin concentrations of sheep in experiment 1. (A) sheep 23 (●) and 27 (■) infected with 150 *F. gigantica* metacercariae, sheep 24 (▲) and 28 (▼) infected with 150 *F. hepatica* metacercariae and uninfected control sheep 21 (O) and 22 (△). (B) sheep 25 (●) and 29 (■) infected with 350 *F. gigantica* metacercariae, sheep 26 (▲) and 30 (▼) infected with 350 *F. hepatica* metacercariae and uninfected control sheep 31 (O) and 32 (△).

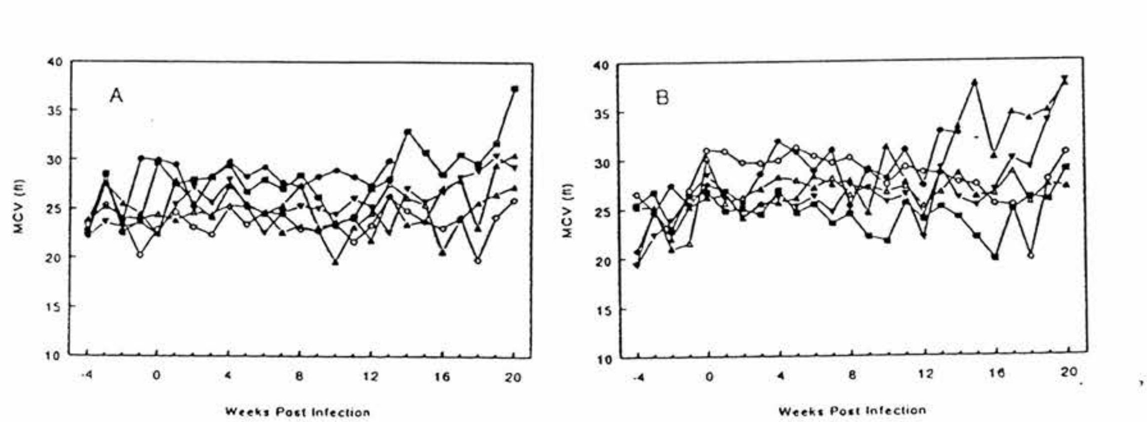


Figure 8.1.11. MCVs of sheep in experiment 1. (A) sheep 23 (●) and 27 (■) infected with 150 *F. gigantica* metacercariae, sheep 24 (▲) and 28 (▼) infected with 150 *F. hepatica* metacercariae and uninfected control sheep 21 (O) and 22 (△). (B) sheep 25 (●) and 29 (■) infected with 350 *F. gigantica* metacercariae, sheep 26 (▲) and 30 (▼) infected with 350 *F. hepatica* metacercariae and uninfected control sheep 31 (O) and 32 (△).

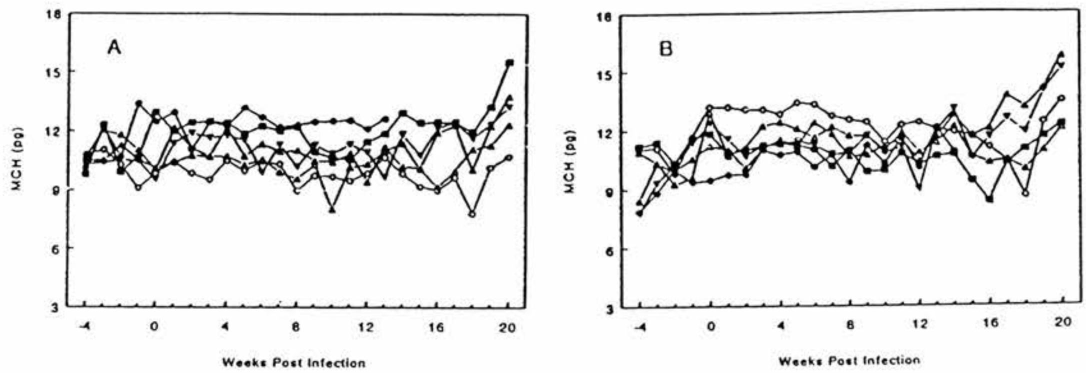


Figure 8.1.12. MCH values of sheep in experiment 1. (A) sheep 23 (●) and 27 (■) infected with 150 *F. gigantica* metacercariae, sheep 24 (▲) and 28 (▼) infected with 150 *F. hepatica* metacercariae and uninfected control sheep 21 (O) and 22 (△). (B) sheep 25 (●) and 29 (■) infected with 350 *F. gigantica* metacercariae, sheep 26 (▲) and 30 (▼) infected with 350 *F. hepatica* metacercariae and uninfected control sheep 31 (O) and 32 (△).

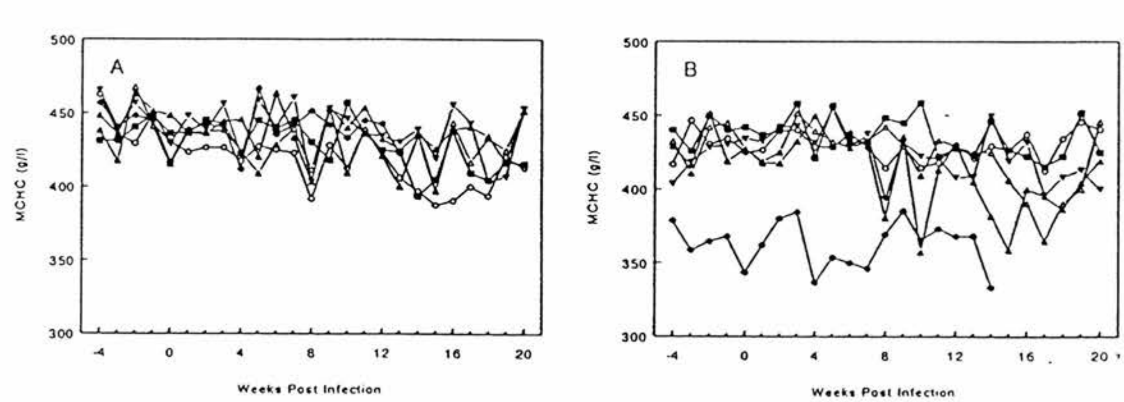


Figure 8.1.13. MCHC values of sheep in experiment 1. (A) sheep 23 (●) and 27 (■) infected with 150 *F. gigantica* metacercariae, sheep 24 (▲) and 28 (▼) infected with 150 *F. hepatica* metacercariae and uninfected control sheep 21 (O) and 22 (△). (B) sheep 25 (●) and 29 (■) infected with 350 *F. gigantica* metacercariae, sheep 26 (▲) and 30 (▼) infected with 350 *F. hepatica* metacercariae and uninfected control sheep 31 (O) and 32 (△).

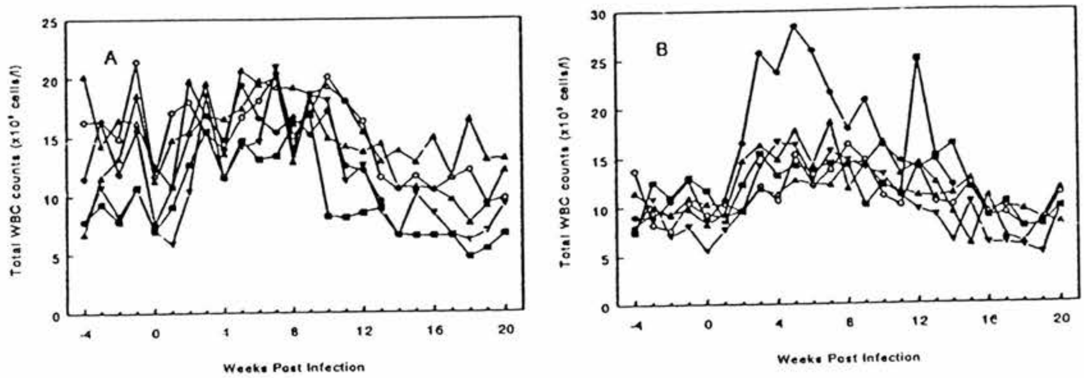


Figure 8.1.14. Total WBC counts of sheep in experiment 1. (A) sheep 23 (●) and 27 (■) infected with 150 *F. gigantica* metacercariae, sheep 24 (▲) and 28 (▼) infected with 150 *F. hepatica* metacercariae and uninfected control sheep 21 (O) and 22 (△). (B) sheep 25 (●) and 29 (■) infected with 350 *F. gigantica* metacercariae, sheep 26 (▲) and 30 (▼) infected with 350 *F. hepatica* metacercariae and uninfected control sheep 31 (O) and 32 (△).

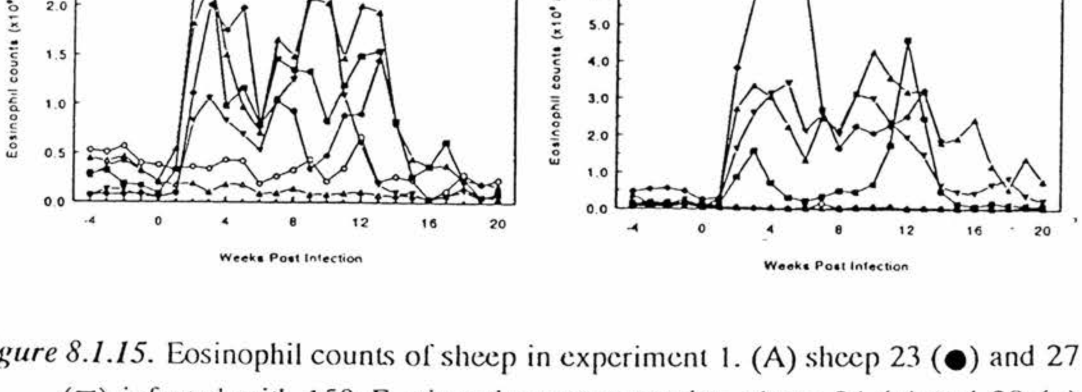


Figure 8.1.15. Eosinophil counts of sheep in experiment 1. (A) sheep 23 (●) and 27 (■) infected with 150 *F. gigantica* metacercariae, sheep 24 (▲) and 28 (▼) infected with 150 *F. hepatica* metacercariae and uninfected control sheep 21 (O) and 22 (△). (B) sheep 25 (●) and 29 (■) infected with 350 *F. gigantica* metacercariae, sheep 26 (▲) and 30 (▼) infected with 350 *F. hepatica* metacercariae and uninfected control sheep 31 (O) and 32 (△).

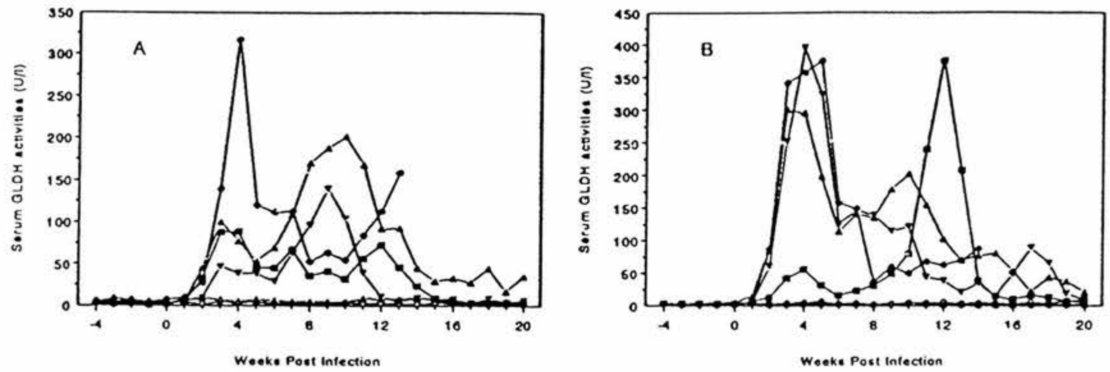


Figure 8.1.16. Serum GLDH levels of sheep in experiment 1. (A) sheep 23 (●) and 27 (■) infected with 150 *F. gigantica* metacercariae, sheep 24 (▲) and 28 (▼) infected with 150 *F. hepatica* metacercariae and uninfected control sheep 21 (O) and 22 (△). (B) sheep 25 (●) and 29 (■) infected with 350 *F. gigantica* metacercariae, sheep 26 (▲) and 30 (▼) infected with 350 *F. hepatica* metacercariae and uninfected control sheep 31 (O) and 32 (△).

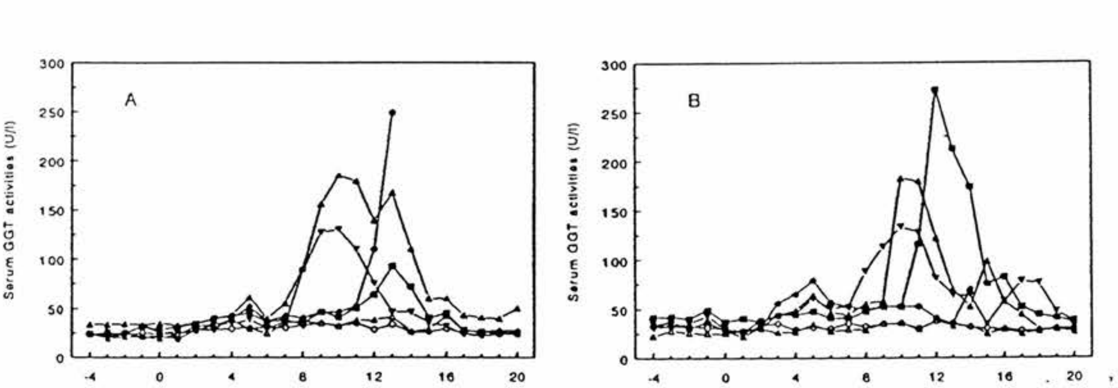


Figure 8.1.17. Serum GGT levels of sheep in experiment 1. (A) sheep 23 (●) and 27 (■) infected with 150 *F. gigantica* metacercariae, sheep 24 (▲) and 28 (▼) infected with 150 *F. hepatica* metacercariae and uninfected control sheep 21 (O) and 22 (△). (B) sheep 25 (●) and 29 (■) infected with 350 *F. gigantica* metacercariae, sheep 26 (▲) and 30 (▼) infected with 350 *F. hepatica* metacercariae and uninfected control sheep 31 (O) and 32 (△).

until 10, 9 and 12 WPI respectively. Sheep 27 and 28 showed a smaller rise than the other infected sheep, and this was followed by an earlier fall to reach the level of the uninfected controls by 15 and 12 WPI respectively. Sheep 29 however showed a sharp rise from 10 WPI reaching a highest level of 375.3 U/l at 12 WPI, but the level fell rapidly during the following 2 weeks.

Although a slight transient rise in serum GGT levels in the infected sheep were recorded between 3 and 5 WPI, this was not pronounced until 8-10 WPI in the sheep infected with *F. hepatica* or 11-12 WPI in the sheep infected with *F. gigantica*, except in the case of sheep 25 where the rise was delayed until 14 WPI (Figure 8.1.17 and Appendix Table 8.1.12). The maximum levels of serum GGT in the *F. hepatica* infected sheep were reached at 10 WPI while those in the *F. gigantica* infected sheep were reached 12-13 WPI. Thereafter, the levels declined gradually and reached to those of uninfected controls by 15, 17 and 18 WPI in sheep 28, 27 and 26 respectively. However, other sheep showed higher levels until the end of the experiment.

8.2 Experiment 2: Experimental *F. gigantica* infection in Nepalese Baruwal sheep

This pilot experiment was designed to produce fasciolosis with burdens of about 150 *F. gigantica* in sheep 11, 12 and 13 (Group A) and about 60 *F. gigantica* in sheep 14, 15 and 16 (Group B). On post mortem examination at 35 WPI, however, only 4 infected sheep (Nos. 11, 14, 15 and 16) were found to harbour flukes, with a burden ranging from 3 to 10. Therefore, the groups of the sheep have been rearranged for analytical purposes as follows:

Group A = infected +ve (sheep 11, 14, 15 and 16),

Group B = infected -ve (sheep 12 and 13) and

Group C = uninfected controls (sheep 17, 18 and 19).

Because of small sample size of the groups, no statistical analysis was done except for regression analysis of liveweights against WPI.

8.2.1 *Clinical data*

During the early preinfection monitoring period, sheep 19 showed signs of anaemia. After infection, however, no clinical signs of fasciolosis were observed in any infected or uninfected sheep.

8.2.2 *Liveweight data*

Except for a transient weight loss during 14 and 15 WPI, the infected sheep grew at a faster rate than the uninfected controls until 20 WPI (Figure 8.2.1 and Appendix Table 8.2.1). During 21 and 22 WPI, all the sheep in the experiment lost weight. Thereafter, however, the uninfected sheep continued to grow at about the previous rate, while the infected sheep grew at a slower rate. Regression analysis of liveweights against WPI (0 to 35 WPI) showed that the mean weight gains in the infected +ve (0.148 kg/week) and infected -ve (0.135 kg/week) sheep were comparatively less than those in the uninfected controls (0.182 kg/week), but these differences were not significant (Appendix Table 8.2.2).

8.2.3 *Carcass data*

Details of the carcass data of the infected and uninfected sheep are shown in Appendix Table 8.2.3. The mean dressing percent (mean weight of the dressed carcass as a percentage of the weight of whole carcass) for the infected +ve (46.7%) and infected -ve (46.0%) sheep were less than that of the uninfected controls (50.7%). Also, less abdominal fat was recorded in the infected +ve (0.675 kg) and infected -ve (0.400 kg) carcasses in comparison to the uninfected controls (0.800 kg).

8.2.4 *Parasitological data*

The prepatent period in the infected +ve sheep ranged from 91 to 105 days (Table 8.2.1). *F. gigantica* eggs were seen in the faeces of sheep 12 for the first time at 161 days after infection. However, the infection never became patent in sheep 13 during the experimental period.

The raw data for *F. gigantica* faecal egg counts are given in Appendix Table 8.2.4 and the weekly mean values (EPG) are shown in Figure 8.2.2. During the first week of the preinfection monitoring (-5 WPI), all sheep had a high number of strongyle eggs in the faeces, ranging from 200 to 3600 EPG, the highest count being in sheep 19. After treatment, however, the counts were low, never exceeding more than 10 EPG.

Table 8.2.1. Parasitological details of the sheep infected with *F. gigantica* in experiment 2.

Sheep	Infective dose (No. of MC)	Prepatent period (days)	Fluke recovery		Mean (± SD) fluke size (mm)	
			No.	%	Length	Width
11	285	91	9	3.2	47.3 (± 3.0)	7.9 (± 0.6)
12	285	161	0	0.0	NA	NA
13	285	**	0	0.0	NA	NA
14	125	105	7	5.6	44.0 (± 3.4)	8.7 (± 0.9)
15	125	105	3	2.4	50.3 (± 1.3)	10.0 (± 0.0)
16	125	91	10	8.0	43.5 (± 4.9)	10.0 (± 0.5)

Note: MC = metacercariae; NA = not applicable; ** = *Fasciola* spp. eggs not detected in this sheep; all sheep were slaughtered at 35 WPI.

As can be seen from table 8.2.1, the recovery of *F. gigantica* was very low, ranging from 0 to 8 %. All the flukes were found in the bile ducts, and their measurements indicated that all were adults. No *Fasciola* spp. were recovered from the uninfected controls. Except for 4 *Eurytrema cladorchis* from the pancreatic ducts of sheep 14, no other helminths were recovered from the rumen, abomasum or intestines of the infected or the uninfected sheep.

8.2.5 *Pathological data*

The livers of the infected +ve and infected -ve sheep were slightly heavier than those of the uninfected controls (Appendix Table 8.2.3). Sheep 16 had an abscess near the main bile duct on the liver surface. The gall bladders of the infected sheep were enlarged and the bile ducts were thickened, even in the infected -ve sheep (Figure 8.2.3).

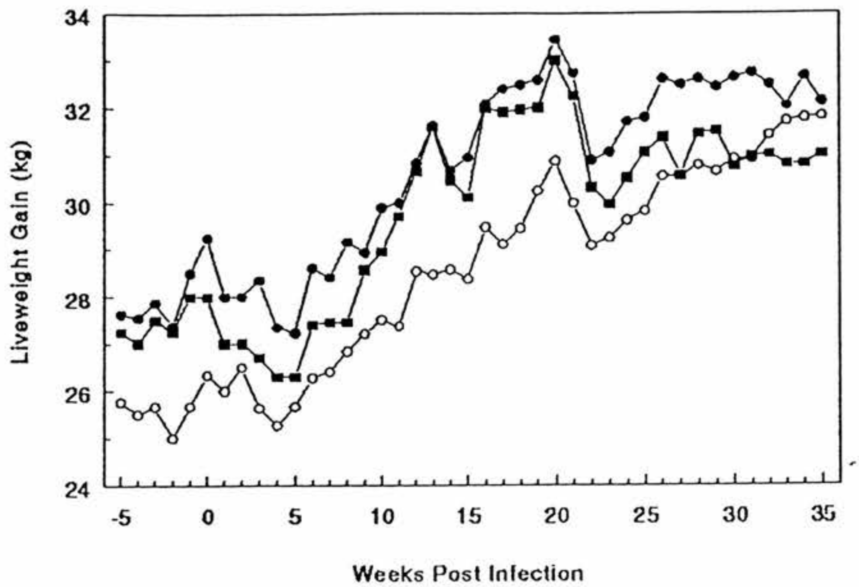


Figure 8.2.1. Mean liveweight of infected +ve (●), infected -ve (■) and uninfected (O) sheep in experiment 2.

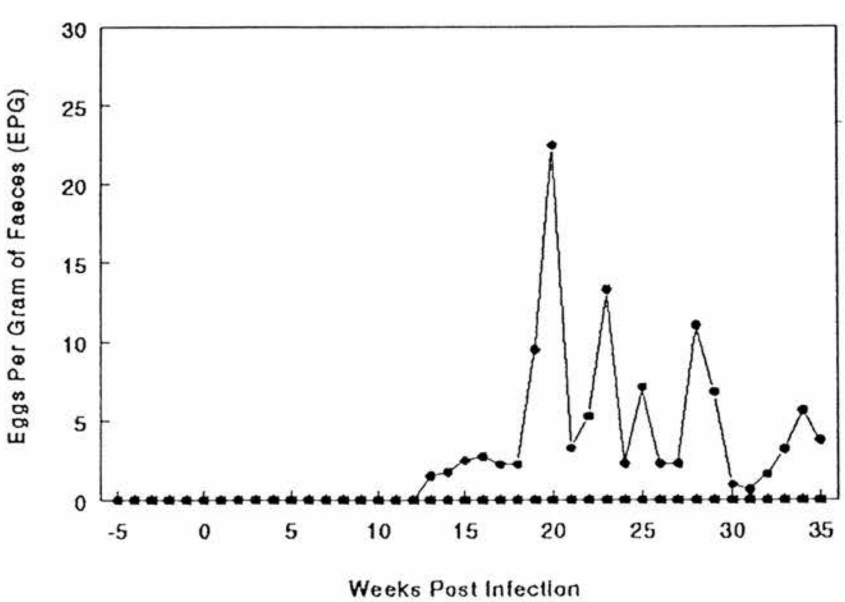


Figure 8.2.2. Mean *F. gigantica* faecal egg counts of infected +ve (●), infected -ve (■) and uninfected (O) sheep in experiment 2.

Histopathological examination of the infected livers (including infected -ve) sections showed most areas to be affected by mild to moderate periportal fibrosis. There was moderate to marked inflammatory cell infiltration periportally. These cells included both mononuclear types (plasma cells, lymphocytes and monocytes) and eosinophils in varying proportions. Some mild biliary hyperplasia was evident. Bile pigments and haemosiderin deposits were evident periportally. With the more marked periportal lesions the adjacent hepatocytes showed degenerative changes (cloudy swelling). The gall bladder and major bile duct mucosa was markedly infiltrated by eosinophils and mononuclear inflammatory cells. Fibrous thickening was apparent beneath the lamina propria and adjacent hepatocytes were degenerated.

8.2.6 *Haematological data*

The RBC counts, packed cell volumes and haemoglobin concentrations are shown in Appendix Tables 8.2.5, 8.2.6 and 8.2.7 respectively. There were no distinct changes in these values as a result of the infection, although slight depressions were observed from 21-22 WPI (Figure 8.2.4).

The total WBC and eosinophil counts are given in Appendix Tables 8.2.8 and 8.2.9 respectively. The total WBC counts did not show any definite pattern (Figure 8.2.5). However, eosinophil counts in the infected sheep rose by 2-3 WPI and remained higher almost until the end of the experiment (Figure 8.2.5). Eosinophilia was bimodal in the infected +ve sheep, with the peaks at 3 and 12 WPI. These peaks were also observed in the infected -ve sheep, however, an additional but higher peak was observed at 22 WPI in sheep 13 and at 28 WPI in sheep 12.

8.2.7 *Biochemical data*

The results of total serum protein and serum albumin determinations are presented in Appendix Tables 8.2.10 and 8.2.11 respectively. No marked changes in total serum protein were observed in the infected sheep, however, the levels which fluctuated above those of the uninfected controls in the beginning appeared to decline after 16-19 WPI (Figure 8.2.6). Similarly, the mean serum albumin levels in the infected sheep declined below those of the uninfected controls from 16 WPI (Figure 8.2.6).

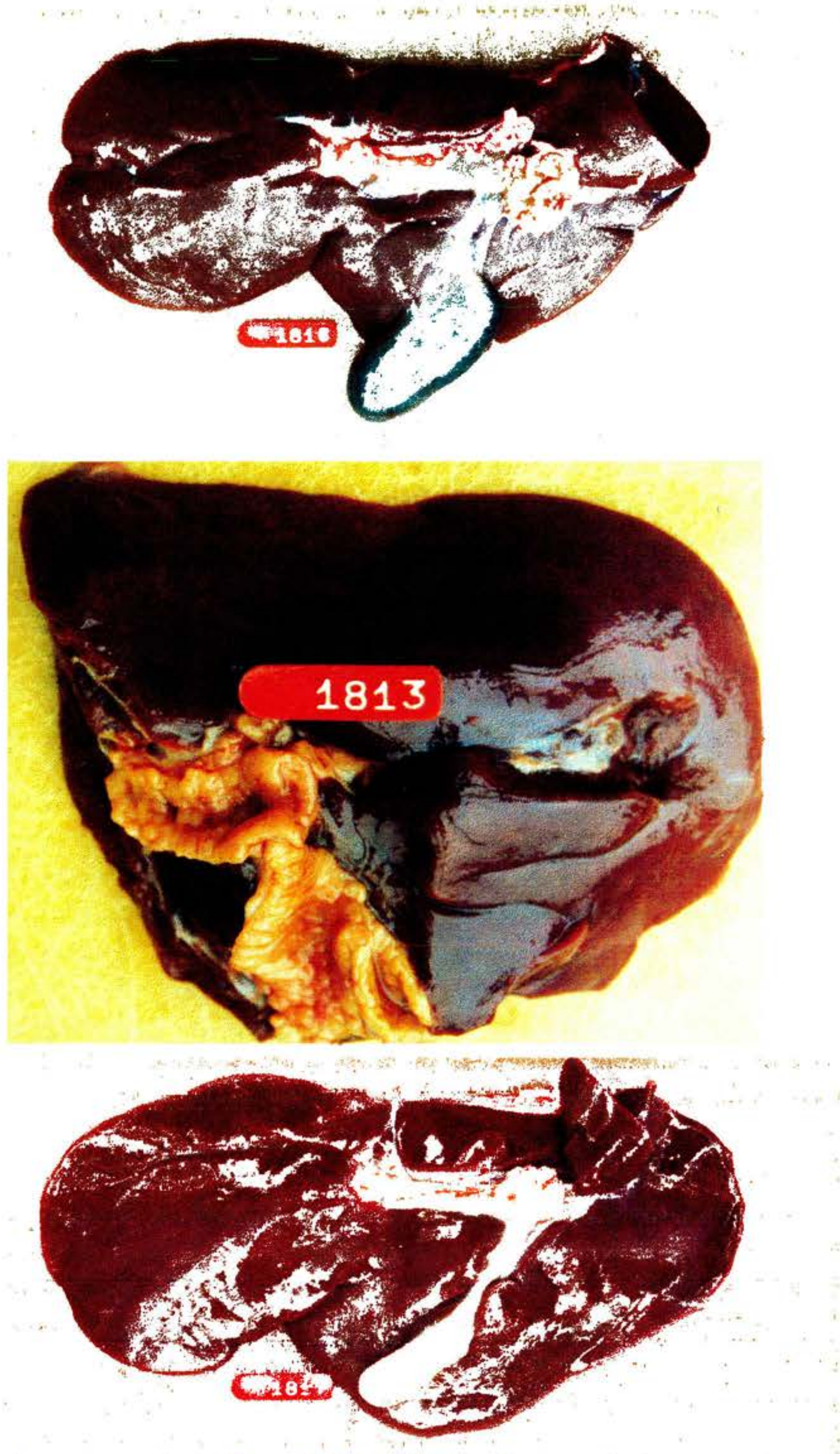


Figure 8.2.3. Liver of sheep 16 with burden of 10 *F. gigantica* showing enlarged gall bladder and an abscess on the surface (top); a portion of the liver of sheep 13 which was given 285 *F. gigantica* metacercariae, but no flukes were recovered on post mortem at 35 WPI showing thickened bile duct (middle); liver of sheep 17, an uninfected control (bottom).

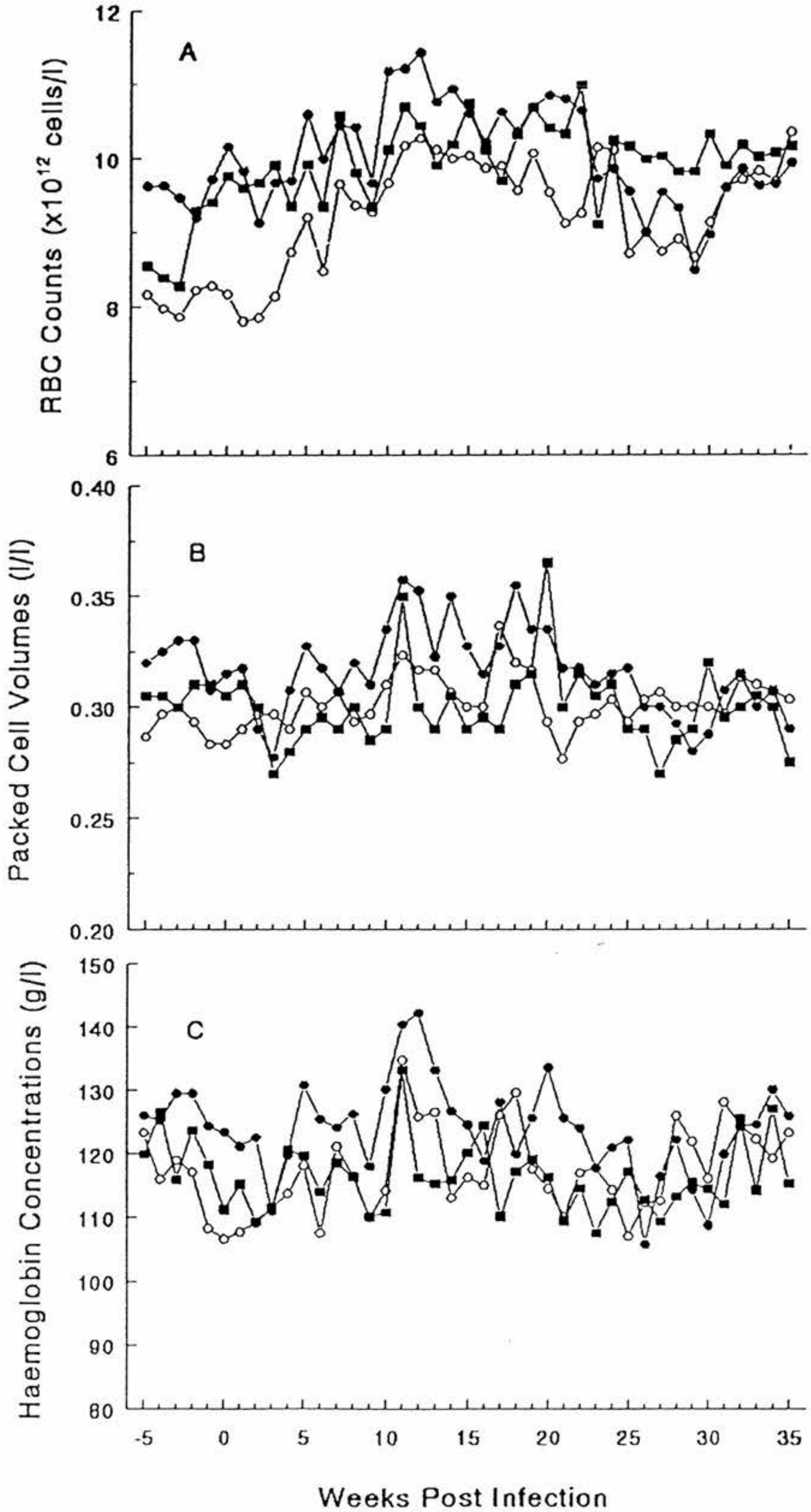


Figure 8.2.4. Mean RBC counts (A), PCVs (B) and haemoglobin concentrations (C) of infected +ve (●), infected -ve (■) and uninfected control (O) sheep.

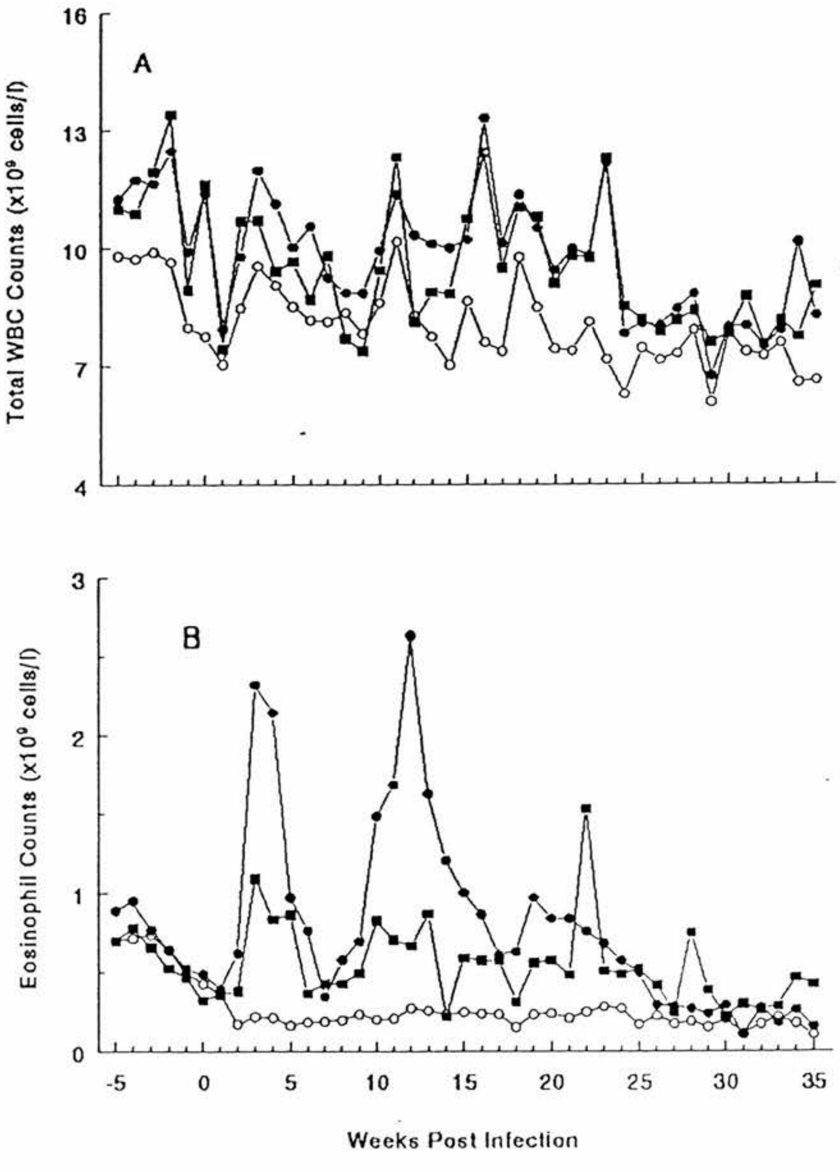


Figure 8.2.5. Mean total WBC (A) and eosinophil counts (B) of infected +ve (●), infected -ve (■) and uninfected control (O) sheep.

The calculated values for serum globulin and serum albumin to globulin ratios are given in Appendix Tables 8.2.12 and 8.2.13 respectively. The levels of serum globulin concentrations in the infected sheep were higher than those of the uninfected controls until 19 WPI (Figure 8.2.7). Although not very marked, the albumin to globulin ratios in the infected sheep were generally lower than the uninfected controls (Figure 8.2.7).

The results of serum GLDH and serum GGT assays are shown in Appendix Tables 8.2.14 and 8.2.15. The serum GLDH values of infected sheep rose by 2-3 WPI (Figure 8.2.8). With several peaks, the values in the infected +ve sheep remained higher than those of the uninfected controls until the end of the experiment while in the infected -ve sheep these dropped to the levels of controls from 31 WPI. The serum GGT levels increased from 8 WPI in the infected +ve and 11 WPI in the infected -ve sheep (Figure 8.2.8). The values in the infected -ve sheep fell below the levels of uninfected controls from 33 WPI, however, these in the infected +ve sheep were elevated until the end of the experiment.

8.2.8 *Agar gel diffusion assay*

Sera from the infected +ve sheep showed precipitin lines from 6-10 WPI (Appendix Table 8.2.16). From 20 WPI, the lines became fainter and after 22 WPI, positive reactions were seen very infrequently. Sera from the infected -ve sheep also showed precipitin lines between 10 and 14 WPI. However, no positive test results were obtained from the uninfected controls at any time during the experiment.

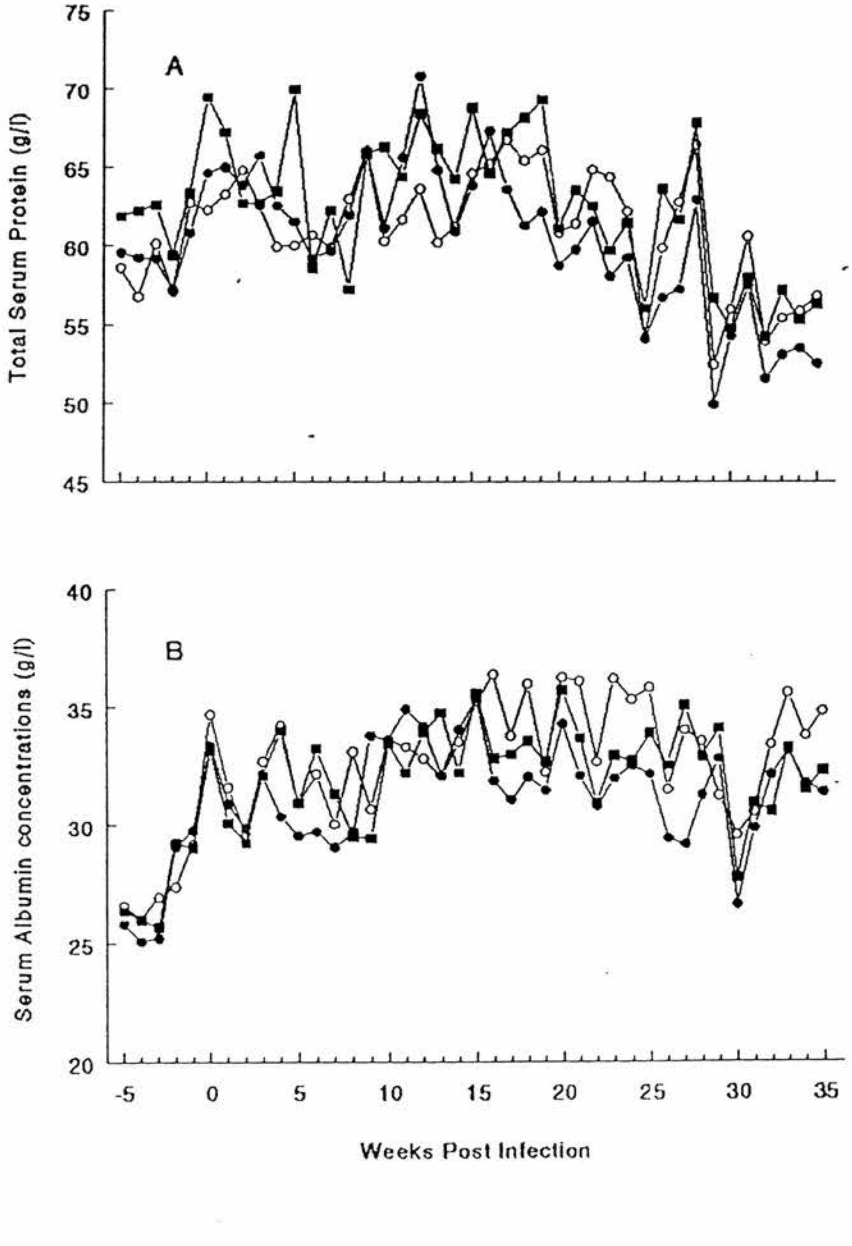


Figure 8.2.6. Mean total serum protein (A) and serum albumin (B) concentrations of infected +ve (●), infected -ve (■) and uninfected control (O) sheep.

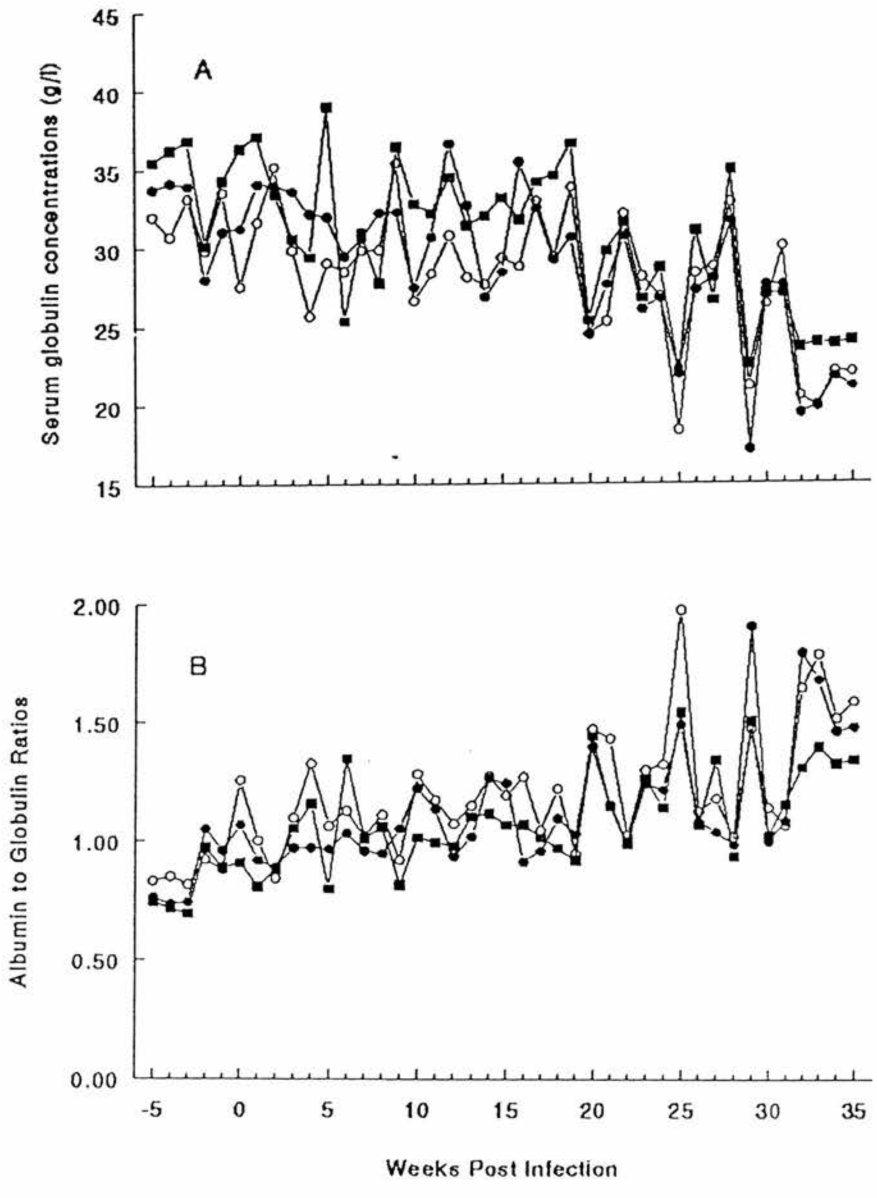


Figure 8.2.7. Mean serum globulin concentrations (A) and albumin to globulin ratios (B) of infected +ve (●), infected -ve (■) and uninfected control (O) sheep.

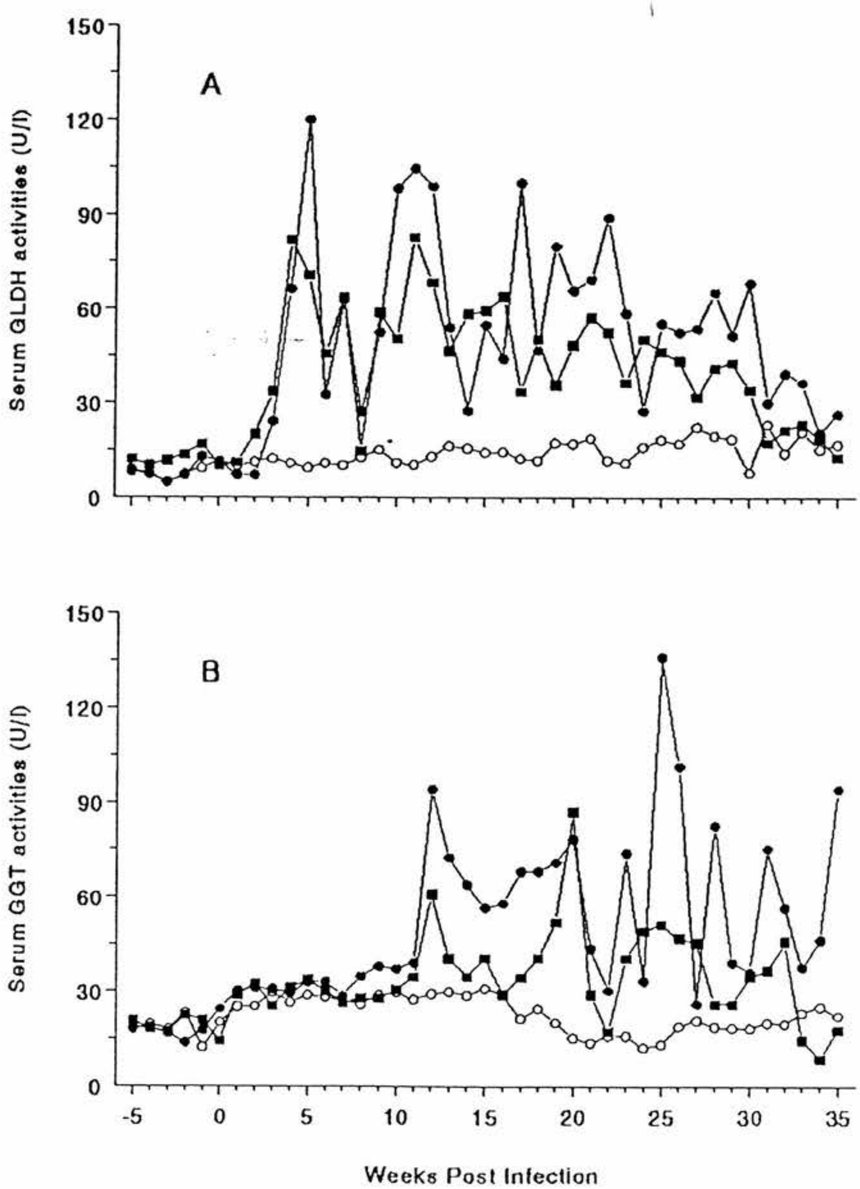


Figure 8.2.8. Mean serum GLDH (A) and GGT (B) levels of infected +ve (●), infected -ve (■) and uninfected control (O) sheep.

8.3 Experiment 3: Experimental *F. gigantica* infection in Nepalese hill goats

8.3.1 *Clinical data*

Among the goats which had received 185 metacercariae (Group A), Nos. 32, 34 and 35 showed periods of inappetence and abdominal discomfort and a rough coat 11 weeks after infection. All these goats became progressively anaemic and weaker (Figure 8.3.1). Listlessness, recumbency and dyspnoea were observed in goat 32 prior to slaughter when moribund at 33 WPI. The remaining goats in this group (Nos. 31 and 36), and none of the goats which had been infected with 80 metacercariae (group B) showed any signs which could be attributed to fasciolosis; moreover, they all appeared to be in good condition throughout the experiment. Similarly, the uninfected controls (group C) showed no sign of any illness during the experimental period.

8.3.2 *Liveweight data*

There was very little difference between the mean liveweights of the three groups until 10 weeks after infection (Figure 8.3.2). Thereafter, the goats infected with 185 metacercariae gained less weight than those given 80 metacercariae and the controls. The goats given 80 metacercariae also appeared to grow at a slower rate compared to that of the controls. However, the Kruskal-Wallis one way analysis of variance showed that the mean liveweights of the three groups were not significantly different at any point during the experiment (Appendix Table 8.3.1).

The mean liveweight gains between 0 and 35 WPI, as calculated from regression analysis were found to be 0.178, 0.378 and 0.439 kg/week for goats given 185, 80 and 0 metacercariae respectively (Appendix Table 8.3.2 and Figure 8.3.3). Analysis of variance on the liveweight gain figures (Appendix Table 8.3.2) showed that there were significant differences between the three groups ($F = 560.0$, d.f. = 2, $P = <0.0001$). Further analysis revealed that liveweight gains of even the goats given 80 metacercariae and controls were significantly different ($F = 43.36$, d.f.= 1, $P = <0.0001$).

Figure 8.3.1. Goats 32, 42 and 52 (from right to left in top plate) with burdens of 55, 15 and 0 and goats 35, 45 and 55 (from right to left in bottom plate) with burdens of 68, 4 and 0 *F. gigantica* respectively, 32 weeks after infection.

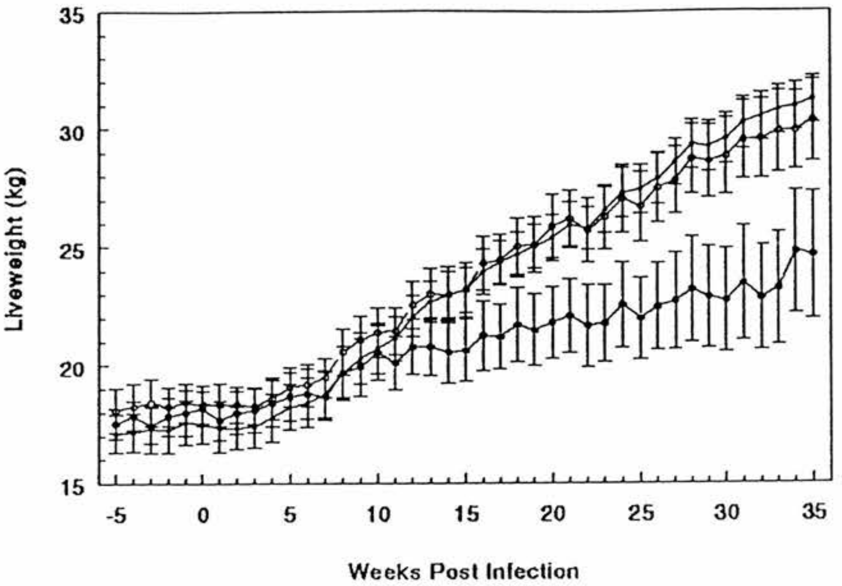


Figure 8.3.2. Mean liveweight ± SE of the goats each infected with 185 (●), 80 metacercariae (■) and uninfected controls (O) in experiment 3.

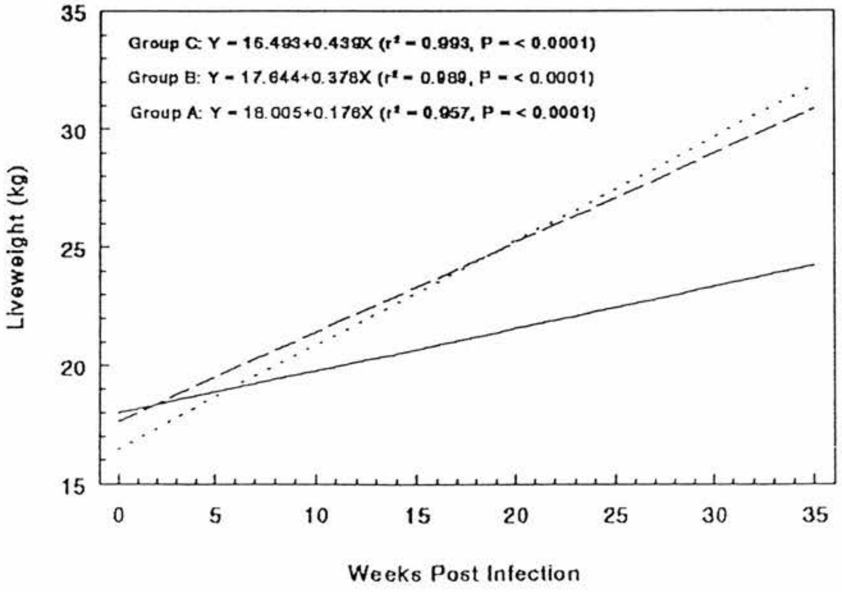


Figure 8.3.3. Regression lines of the mean liveweights of goats given 185 metacercariae or groups A (—), 80 metacercariae or group B (--) and uninfected controls or group C (••). Slopes of these lines are significantly different ($F = 560$, d.f. $= 2$, $P = < 0.0001$).

There was a significant negative correlation between liveweight gain and terminal fluke burden ($r = -0.885$, d.f. = 16, $P = < 0.0001$) (Figure 8.3.4). The regression suggested that each fluke depressed the liveweight gain of the infected goats by about 6.0 g per week.

8.3.3 *Carcass data*

After post mortem examination, carcasses of the infected and uninfected goats were processed for the estimation of dressing percentage. This is defined as the weight of dressed carcass (including shaved skin and edible offal) as a percentage of the weight of whole carcass. The carcass data for the individual goats together with the results of statistical comparisons between the groups are given in Appendix Table 8.3.4.

The mean weights of the dressed carcasses for the goats given 185 and 80 metacercariae were 31.1 and 5.1% less than those for the uninfected controls respectively. One-way analysis of variance (ANOVA) showed that there were significant differences between the mean carcass weights of the three groups ($F = 5.11$, d.f. = 2, $P = 0.0203$). As located by post-tests, these differences were between the groups A and B ($P = 0.0320$) and A and C ($P = 0.0162$) but not between B and C ($P = 0.6990$).

The mean dressing percent of 45.3 for the group A, 49.2 for the group B and 50.1 for the group C were significantly different, however the differences lay only between the groups A and C ($P = < 0.0280$). In comparison to the groups B or C, group A had significantly less abdominal fat ($P = 0.0181$ and 0.0348 respectively) which can be clearly seen in Figure 8.3.6. However, there was no significant difference between the mean weight of abdominal fat for the groups B and C ($P = 0.7121$).

Dressing percent and weight of abdominal fat of the goats were found to be negatively correlated with terminal fluke burden ($r = -0.878$, d.f. = 16, $P = < 0.0001$ and $r = -0.775$, d.f. = 16, $P = < 0.0001$ respectively).

8.3.4 *Parasitological data*

The mean prepatent period for *F. gigantica* infections was 90.2 ± 4.3 days for the goats given 185 metacercariae and 100.0 ± 10.9 days for the goats given 80 metacercariae. There was a tendency for the prepatent period to be shorter in the heavier infections (Table 8.3.1). The highest mean *F. gigantica* faecal egg counts of 24.3 EPG for the goats given 185 metacercariae and only 3.9 EPG for the goats given 80 metacercariae were observed at 20 and 35 WPI respectively and there appeared to be no exponential rise in EPG in these goats (Figure 8.3.5). There was a significant difference between mean EPG of the two infected groups from 14 WPI until the end of the experiment (Appendix Table 8.3.5) and the mean EPG correlated with terminal fluke burden ($r = 0.889$, d.f. = 10, $P = < 0.001$).

Table 8.3.1. Some parasitological details of the infected goats in experiment 3.

Group	Goat No.	Prepatent period (days)	Fluke recovery No.	Fluke recovery %	Mean fluke size in mm (± SD) Length	Mean fluke size in mm (± SD) Width
A (185 MC)	31	91	4	2.2	51.6 (± 7.0)	10.0 (± 0.6)
	32	87	55	29.7	41.2 (± 3.8)	8.8 (± 1.0)
	33	90	23	12.4	42.8 (± 3.7)	9.5 (± 0.8)
	34	88	45	24.3	42.3 (± 3.6)	9.4 (± 0.7)
	35	86	68	36.8	38.4 (± 2.5)	9.1 (± 0.8)
	36	99	7	3.8	45.8 (± 6.4)	9.5 (± 1.0)
	Mean	90.2	33.7	18.2	44.03	9.37
B (80 MC)	41	90	4	5.0	43.4 (± 3.8)	9.4 (± 0.8)
	42	114	15	18.8	42.3 (± 1.9)	9.6 (± 0.5)
	43	91	12	15.0	41.3 (± 3.2)	9.2 (± 0.9)
	44	116	5	6.3	42.4 (± 3.2)	9.2 (± 0.9)
	45	91	4	5.0	44.7 (± 3.8)	9.1 (± 1.0)
	46	98	14	17.5	41.2 (± 4.6)	8.6 (± 1.0)
	Mean	100.0	9.0	11.3	42.53	9.17

Goat 32 died 33 weeks after infection, the remaining goats were slaughtered 35 weeks after infection. MC denotes infective dose (number of metacercariae given to each goat).

The experiment was terminated 35 weeks after infection but goat 32 died 33 WPI. All the *Fasciola* found were adults and in the bile ducts. Spearman's rank correlation coefficient indicated that there was a negative correlation between the mean fluke length and fluke burden at the end of the experiment ($r_s = -0.7587$, d.f. = 10, $P = 0.004$). The numbers of flukes recovered from each infected goat expressed as a percentage of the infecting dose of metacercariae are shown in Table 8.3.1. Recovery ranged from 2.2 to 36.8% with a mean of 18.2% for goats given 185 metacercariae and 5.0 to 18.8% with a mean of 11.3% for goats given 80 metacercariae. No *Fasciola* spp. were recovered from the uninfected controls.

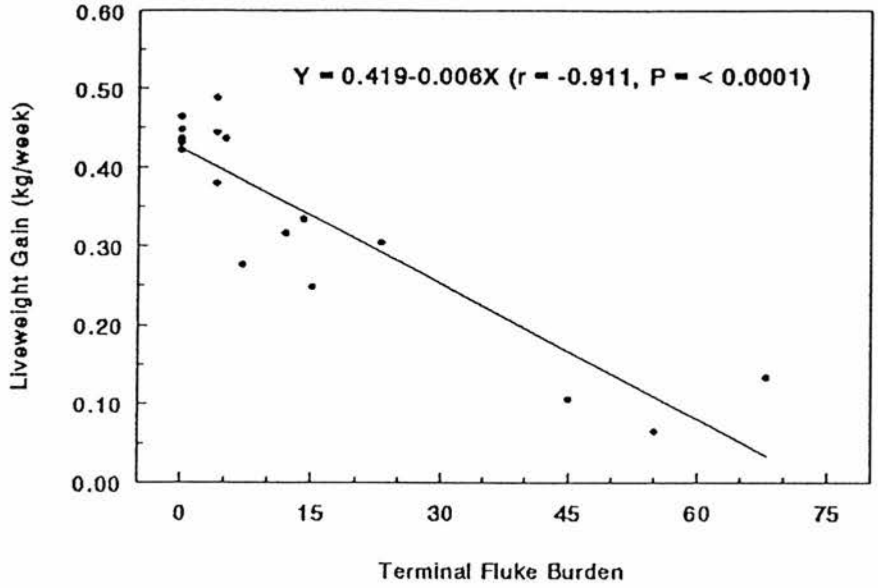


Figure 8.3.4. Regression line showing the relationship between the fluke burden and liveweight gain in goats in experiment 3.

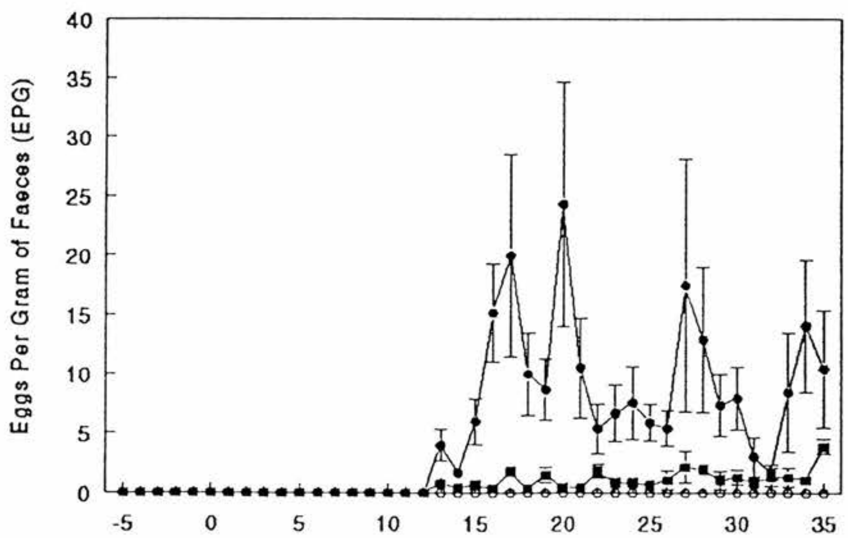


Figure 8.3.5. Mean *F. gigantica* faecal egg counts ± SE of the goats each infected with 185 (●) and 80 metacercariae (■) and uninfected controls (O) in experiment 3.

Two *Moniezia expansa* were recovered from the small intestines of goats 42 and 45, 4 *Taenia hydatigena* metacestodes (*Cysticercus tenuicollis*) recovered from the peritoneum of goats 32 and 41. Small numbers (< 10) of *Haemonchus* spp. were also recovered from the abomasum of some goats.

8.3.5 *Pathological data*

The carcasses of the heavily infected goats (Nos. 32, 34 and 35) were pale and there was atrophy of mesenteric fat (Figure 8.3.6). The livers were enlarged with rounded borders and tougher than normal in consistency. The livers of the remaining infected goats which had mild infections were apparently normal in colour and appearance. In all the infected goats, the bile ducts were distended with thickened walls, but the gall bladders were not enlarged (Figure 8.3.7).

Histopathological examination of livers sections showed mild to moderate periportal fibrosis with some mild biliary hyperplasia. The periportal inflammatory cell infiltrate was mild to marked and comprised both mononuclear cells and eosinophils, the later predominating in the more florid lesions (Figure 8.3.8). Similarly, the degree of periportal hepatocyte degeneration was directly related to the extent of the lesions with fatty degeneration evident in some regions. Pigmentary deposits, such as bile and haemosiderin were evident periportally (Figure 8.3.8). The gall bladder and major bile duct lamina propria were infiltrated by many mononuclear inflammatory cells and eosinophils, and increased fibrosis was apparent around these structures.

8.3.6 *Haematological data*

In infected goats, there was a reduction in RBC counts, PCVs and haemoglobin concentrations beginning 10 to 12 weeks after infection while the uninfected goats maintained their original levels (Figure 8.3.9). These values did not declined progressively in the infected goats as there were several transient rises after initial fall, however they remained lower than of the control goats. The Kruskal-Wallis one way analysis of variance indicated that the RBC counts of the three groups were significantly different only at 30 WPI, whereas the differences in PCVs

***Figure** 8.3.6.* Carcasses of goats 35, 45 and 55 (from left to right) with burdens of 68, 4 and 0 *F. gigantica* respectively. The comparatively paler carcass of goat 32 had no fat around the kidneys and also less mesenteric fat (displayed here behind the tail).

***Figure** 8.3.7.* Livers of goats 35, 45 and 55 (from left to right) with burdens of 68, 4 and 0 *F. gigantica* respectively. The bile ducts in both the infected goats were thickened and were also distended in goat 35.

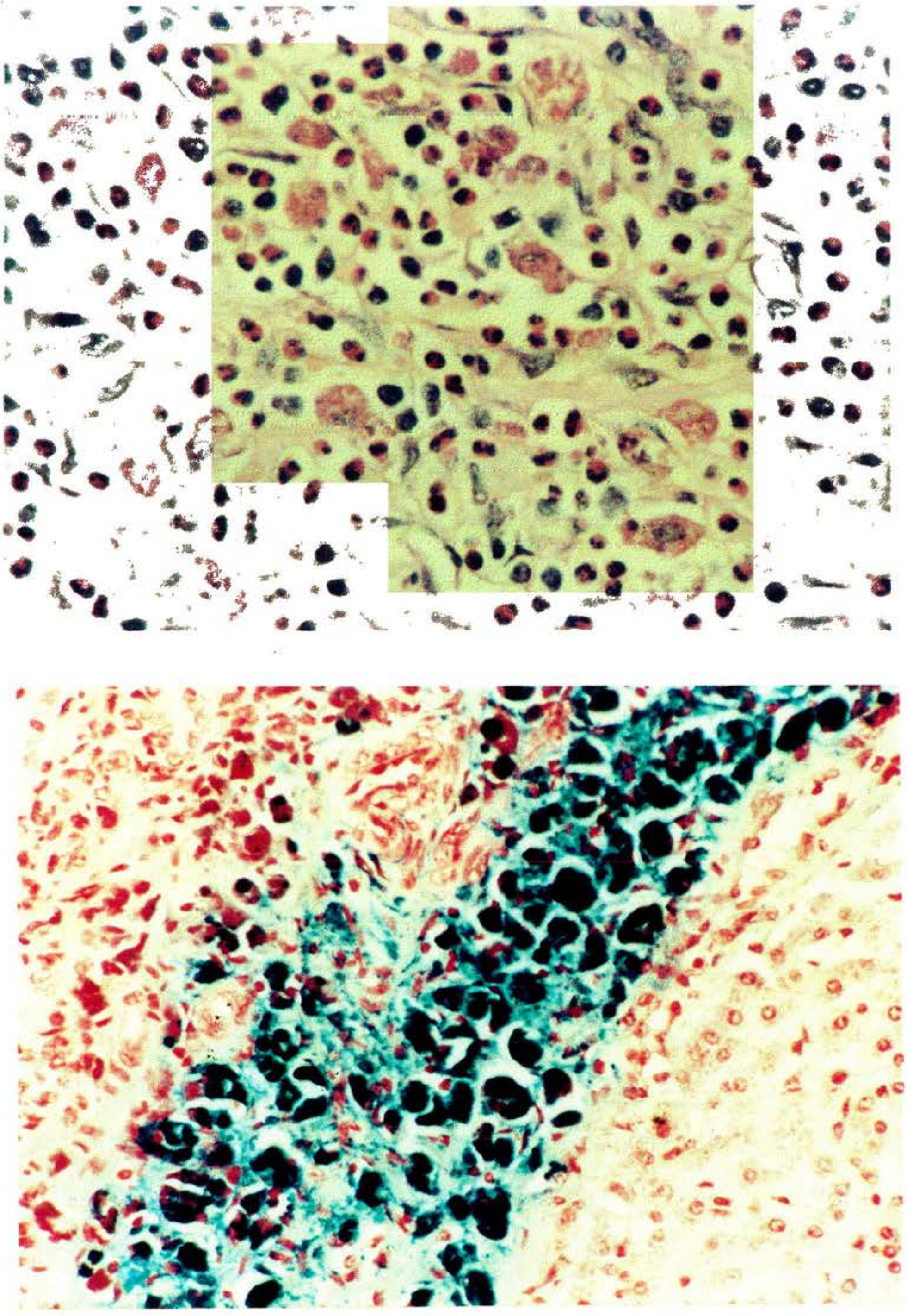


Figure 8.3.8. Top plate: details of a periportal area (goat 33 with burden of 23 *F. gigantica*, slaughtered 35 WPI) showing many eosinophils amongst inflammatory cell infiltrates (stained with CC, magnification x520); Bottom plate: haemosiderin deposits in a periportal area of goat 32 with burden of 55 *F. gigantica*, died 33 WPI (stained with PBR, magnification x520).

and haemoglobin concentrations were significant from 13 and 15 WPI respectively until end of the experiment (Appendix Tables 8.3.6, 8.3.7 and 8.3.8). Further analysis using the Mann-Whitney test revealed that the differences in RBC counts lay between the groups A and B, and A and C but not between B and C (Table 8.3.2). However, for PCVs and haemoglobin concentrations, the differences were located between all the three groups (Table 8.3.2). Regressions of the mean RBC counts, PCVs and haemoglobin concentrations (from 12 to 35 WPI) on terminal fluke burden showed significant negative correlations (r = -0.886, d.f. = 10, P = < 0.001; r = -0.894, d.f. = 10, P = < 0.001; r = -0.905, d.f. = 10, P = < 0.001 respectively).

There were no changes in MCV values as a result of the infection (Figure 8.3.10 and Appendix Table 8.3.9). In general, the levels of MCH and MCHC values in the infected goats were depressed (Figure 8.3.10), although, the differences were significant only at 35 and 25 WPI respectively (Appendix Tables 8.3.10 and 8.3.11). As indicated by the Mann-Whitney test, the difference in MCH was significant only between the groups A and C (Table 8.3.2). MCHC values of the control goats (group C) differed significantly with those of the both infected groups, however, there was no significant difference between the two infected groups (Table 8.3.2).

Table 8.3.2. Statistical comparisons of the haematological indices using the Mann-Whitney test to locate the differences between the three groups of goats in experiment 3.

Parameters	Data points (n)	Group A vs B		Group A vs C		Group B vs C	
		U	P	U	P	U	P
RBC Counts	6/6	3.5	0.0152	2.0	0.0087	14.5	0.5887
Packed cell volumes	23/23	0.5	<0.0001	0.0	<0.0001	110.0	0.0007
Hb concentrations	21/21	0.0	<0.0001	0.0	<0.0001	16.5	<0.0001
MCH	5/6	5.0	0.0823	3.0	0.0303	10.0*	0.2403
MCHC	6/6	15.0	0.6991	4.0	0.0260	2.0	0.0087
Eosinophil counts	23/23	36.0	<0.0001	0.0	<0.0001	82.5	<0.0001

* Data points for this comparison = 6/6.

There were no significant changes in total WBC counts of the infected goats (Appendix Table 8.3.12), although the levels, particularly those of the group A were generally higher than the uninfected controls (Figure 8.3.11). Eosinophil counts in the both groups of infected goats rose by the 2nd week after infection. Several rises and

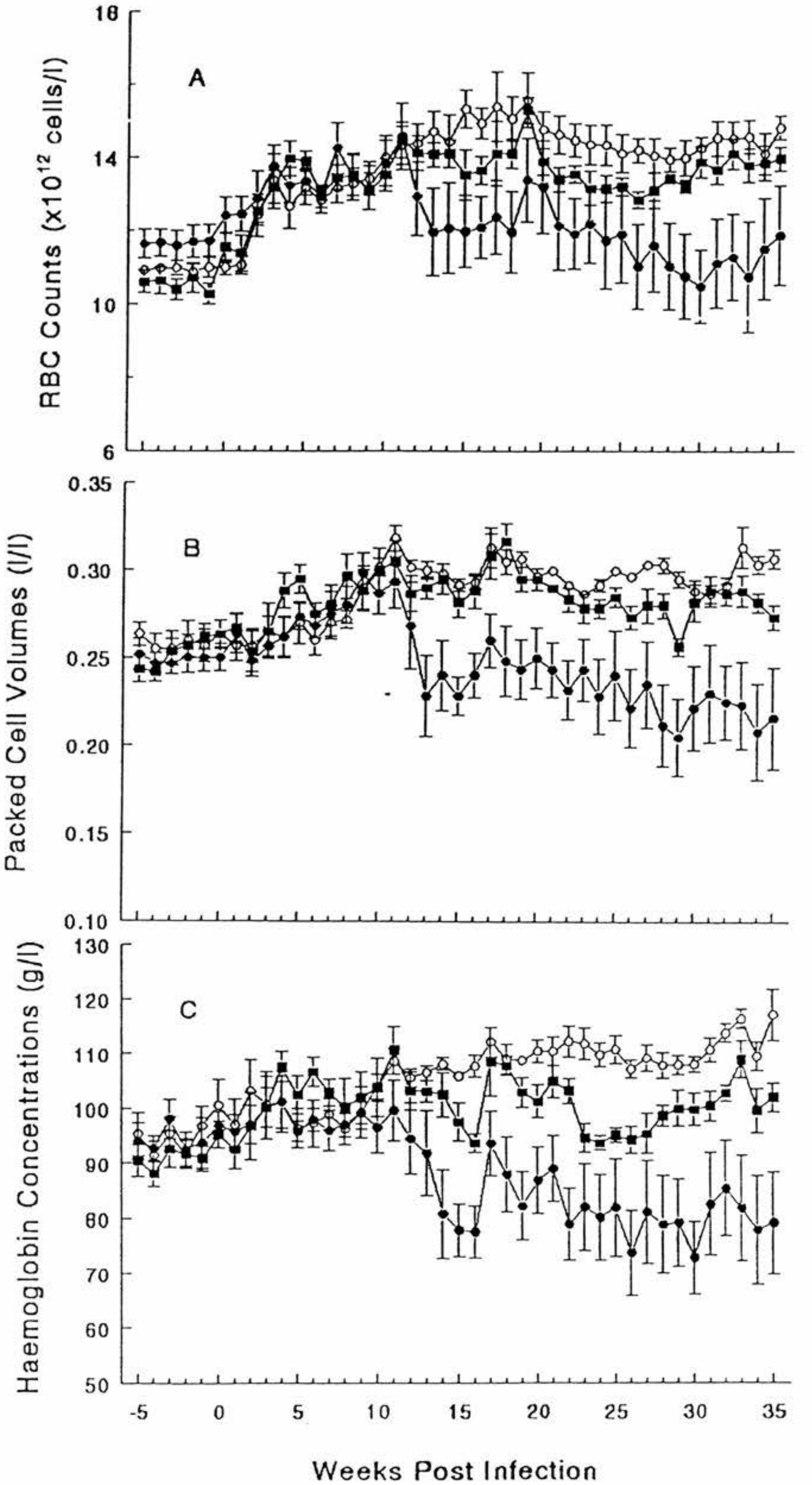


Figure 8.3.9. Mean values ± SE of RBC counts (A), PCVs (B) and haemoglobin concentrations (C) of the goats infected with 185 (●) or 80 metacercariae (■) and uninfected controls (O).

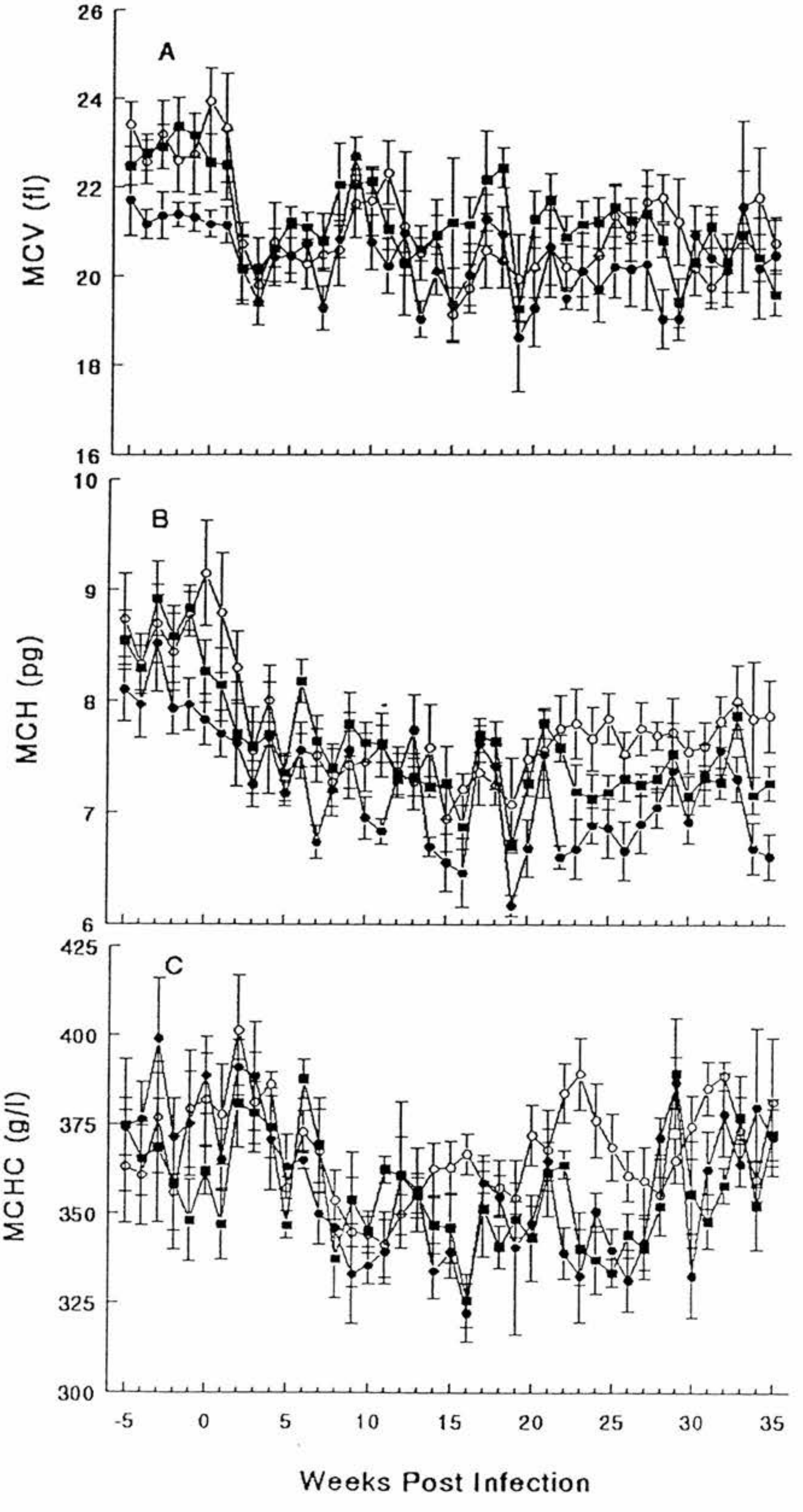


Figure 8.3.10. Mean values ± SE of total MCV (A), MCH (B) and MCHC (C) of the goats infected with 185 (●) or 80 metacercariae (■) and uninfected controls (O).

falls occurred thereafter, with the highest peak at 13 WPI in goats given 185 metacercariae and 14 WPI in goats given 80 metacercariae (Figure 8.3.11). The differences, which were observed between all the three groups (Table 8.3.2) were significant from 2 until 24 WPI (Appendix Table 8.3.13). There was significant positive correlation between mean eosinophil counts and terminal fluke burden ($r = 0.737$, d.f. = 10, $P = 0.006$).

8.3.7 *Biochemical data*

There appeared to be a slight rise in the mean total serum protein levels of the infected goats between 11 and 16 WPI, however the levels declined thereafter, particularly in goats given 185 metacercariae (Figure 8.3.12). The Kruskal-Wallis one way analysis of variance showed that there were significant differences in total serum protein concentrations of the three groups of goats from 30 WPI until the end of the experiment (Appendix Table 8.3.14) and the differences were observed between all the groups (Table 8.3.3). There was a significant negative correlation between mean total serum protein concentration (from 17 to 35 WPI) and fluke burden ($r = -0.833$, d.f. = 10, $P = 0.001$).

Table 8.3.3. Statistical comparisons of the biochemical constituents using the Mann-Whitney test to locate the differences between the three groups of goats in experiment 3.

Parameters	Data points (n)	Group A vs B		Group A vs C		Group B vs C	
		U	P	U	P	U	P
Total serum protein	6/6	0.0	0.0022	0.0	0.0022	0.0	0.0022
Serum albumin	22/22	38.0	<0.0001	7.0	<0.0001	153.0	0.0380
GLDH	27/27	269.0	0.0492	0.0	<0.0001	1.0	<0.0001
GGT	18/18	40.0	0.0001	0.0	<0.0001	0.0	<0.0001

There were declines in the mean serum albumin concentrations of the infected goats from 13 WPI which were more pronounced in the group A (Figure 8.3.12). The Kruskal-Wallis test indicated that the differences were significant from 14 WPI till termination of the experiment (Appendix Table 8.3.15). The Mann-Whitney test revealed that the differences lay between all the three groups (Table 8.3.3). There was a significant negative correlation between mean serum albumin concentration (from 14 to 35 WPI) and fluke burden ($r = -0.871$, d.f. = 10, $P = < 0.0001$).

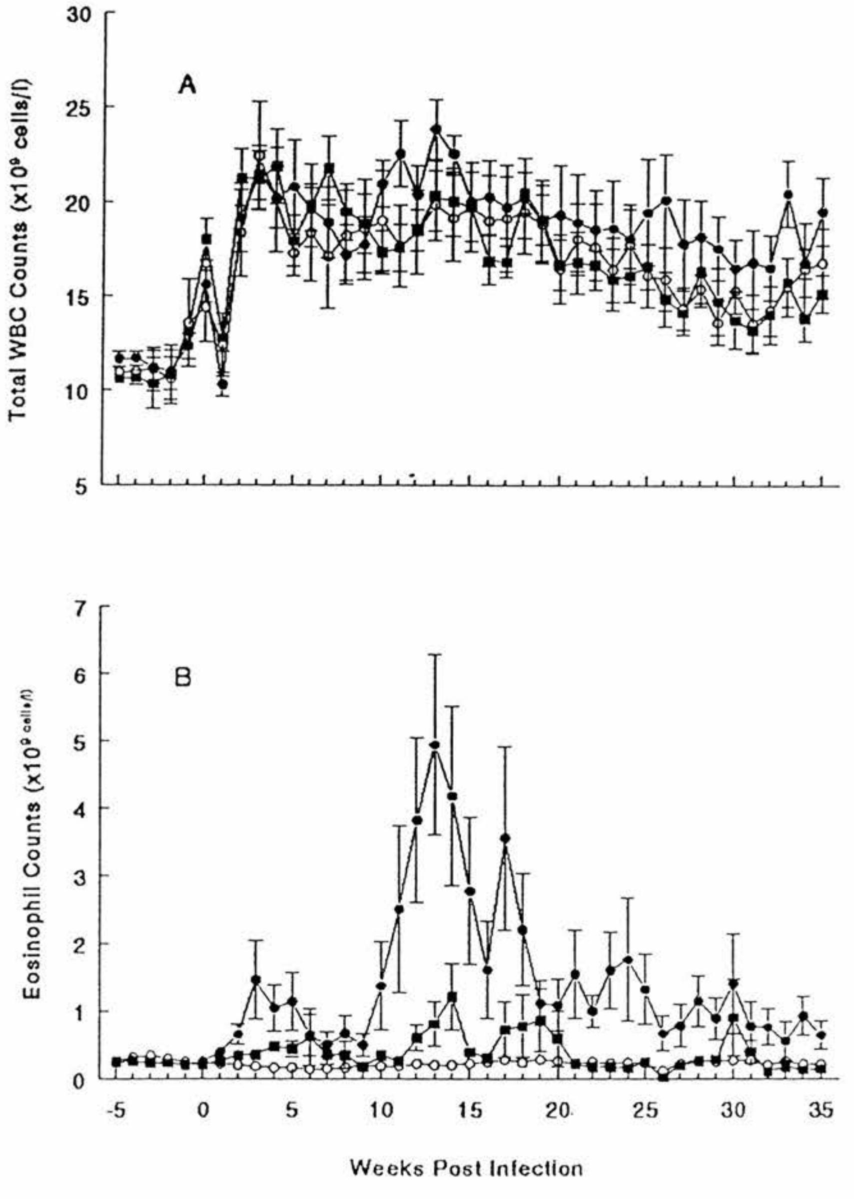


Figure 8.3.11. Mean values ± SE of total WBC (A) and eosinophil counts (B) of the goats infected with 185 (●) or 80 metacercariae (■) and uninfected controls (O).

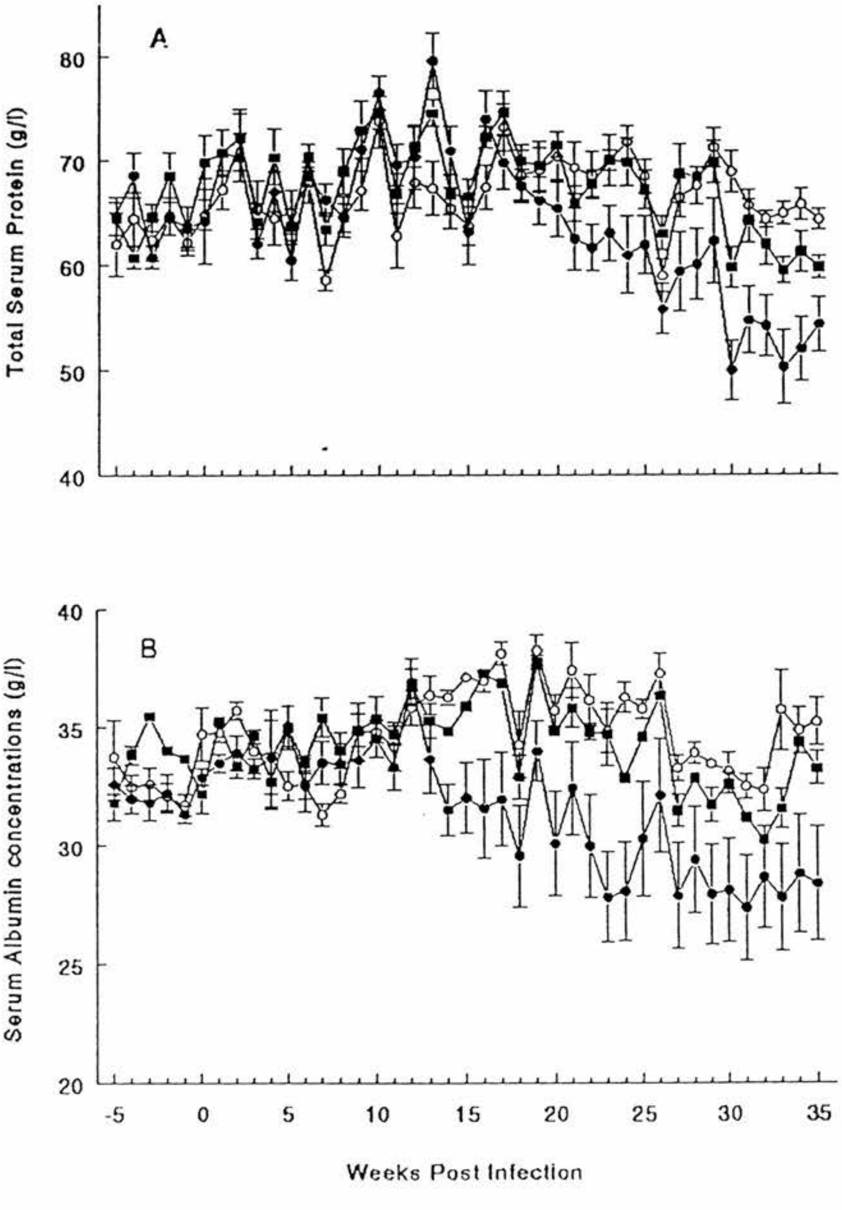


Figure 8.3.12. Mean values ± SE of total serum protein (A) and serum albumin concentrations (B) of the goats infected with 185 (●) or 80 metacercariae (■) and uninfected controls (O).

Infected goats exhibited transient rises in serum globulin concentrations between 13 and 18 WPI. However, the levels declined from 21 WPI in goats given 185 metacercariae and 30 WPI in goats given 80 metacercariae (Figure 8.3.13). The differences in the serum globulin concentrations between the groups were not significant (Appendix Table 8.3.16). The serum albumin to serum globulin ratios were generally lower in the infected compared to the uninfected goats (Figure 8.3.13). These declines were more marked between 13 and 18 WPI, however, the differences were not significant at any point during the experiment (Appendix Table 8.3.17).

The mean serum GLDH levels in both groups of infected goats rose by the 4th week after infection and reached a peak by 5 WPI (Figure 8.3.14). A second and more pronounced peak was observed at 11 WPI in goats given 185 metacercariae and at 12 WPI in goats given 80 metacercariae. Thereafter, the levels slowly declined to those of the uninfected controls by 31 WPI. Statistical analysis indicated that the differences were significant between 4 and 30 WPI (Appendix Table 8.3.18 and Table 8.3.3). There was a significant positive correlation between mean GLDH level (from 4 to 30 WPI) and fluke burden ($r = 0.652$, d.f. = 10, $P = 0.020$).

There were sharp rises in serum GGT levels in the infected goats from 11 WPI (Figure 8.3.14). The highest peak was reached at 13 WPI in goats given 185 metacercariae and at 14 WPI in goats given 80 metacercariae. Thereafter, the levels fell sharply, however they remained higher than those of the uninfected control until end of the experiment. There were significant differences between the groups from 12 to 29 WPI (Appendix Table 8.3.19). The Mann-Whitney test revealed that these differences lay between all the three groups (Table 8.3.3). There was marginally significant correlation between mean GGT levels (from 12 to 29 WPI) and terminal fluke burden ($r = 0.560$, d.f. = 10, $P = 0.056$).

8.3.8 *Agar gel diffusion assay*

Sera from the most heavily infected goats (Nos. 32, 34 and 35) in group A showed precipitin lines from 4-5 WPI until death or termination of the experiment (Appendix Table 8.3.20). Precipitin lines in the sera from lightly infected goats were frequently absent or very faint. Sera from all the uninfected controls gave negative test results during the whole experimental period.

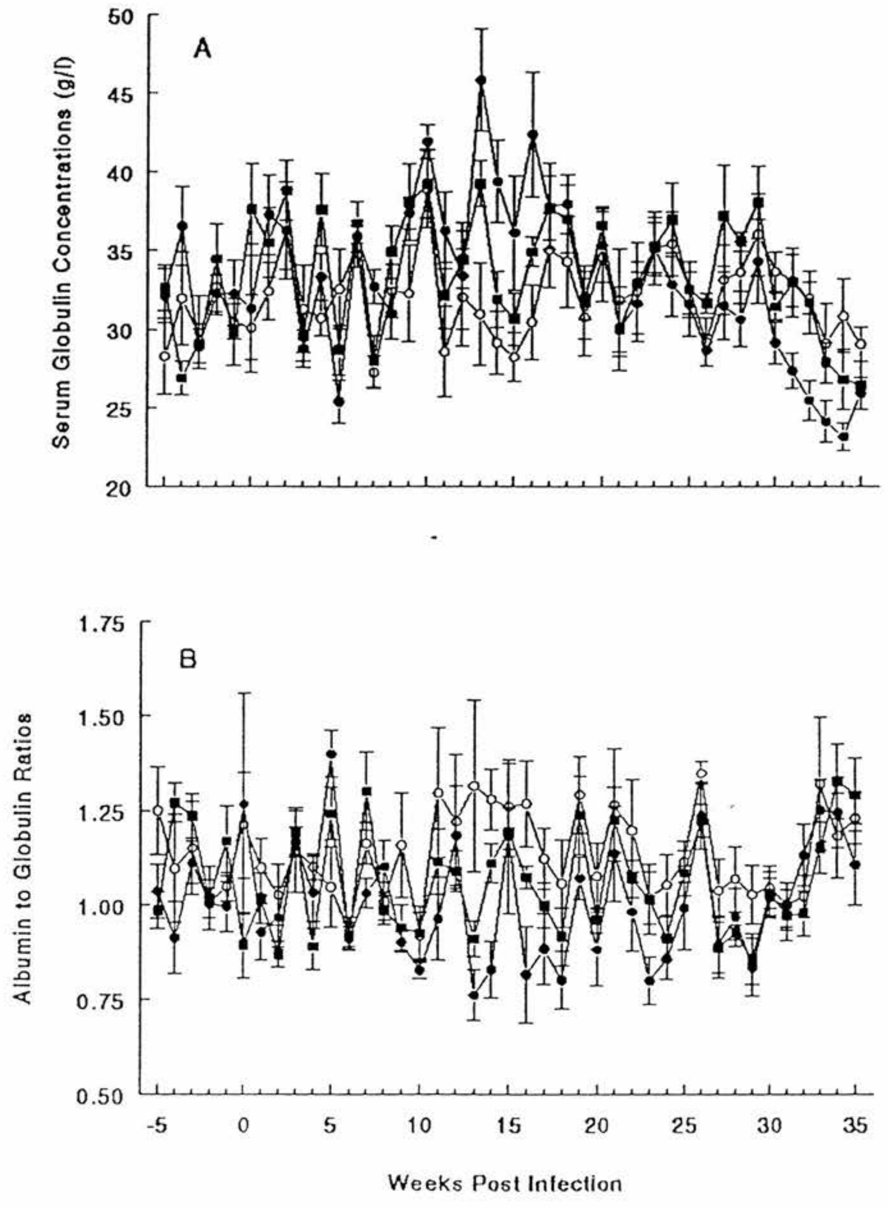


Figure 8.3.13. Mean values ± SE of serum globulin (A) and albumin to globulin ratios (B) of the goats infected with 185 (●) or 80 metacercariae (■) and uninfected controls (O).

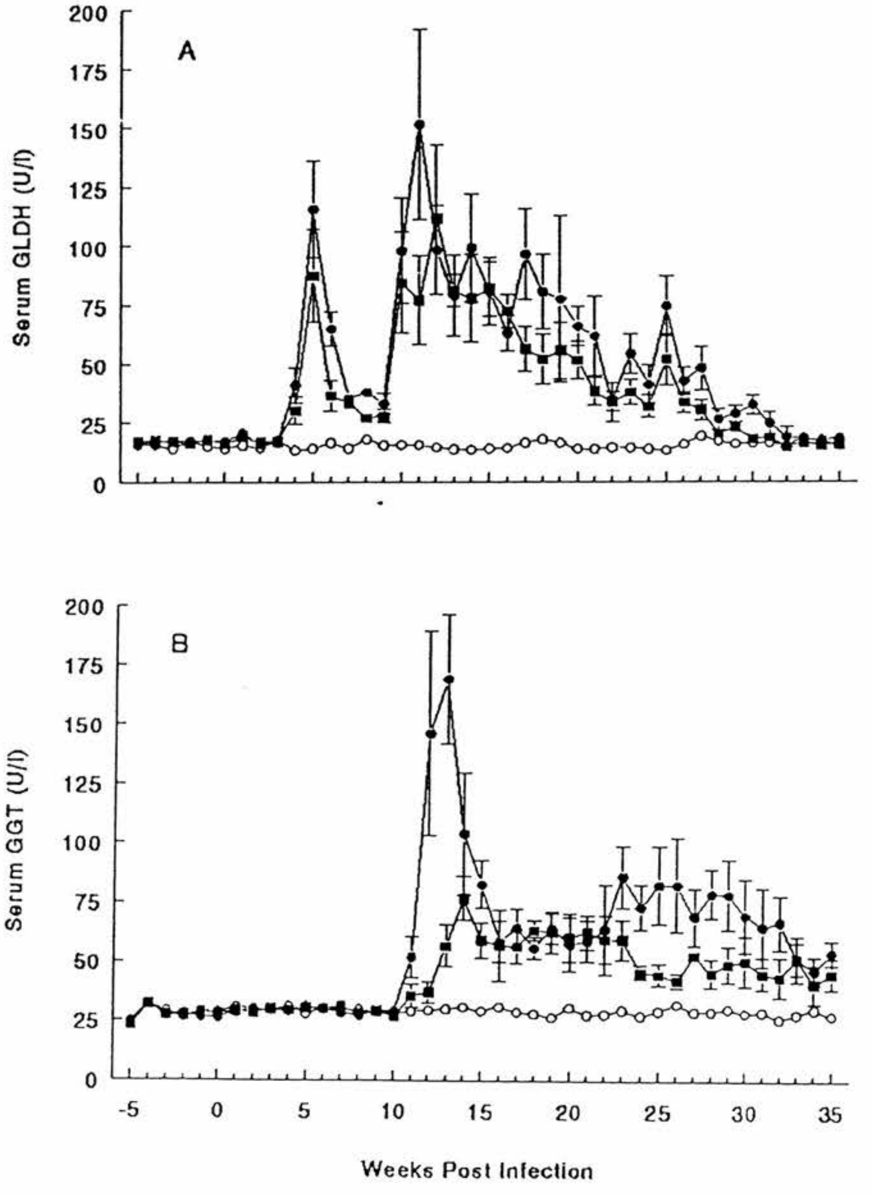


Figure 8.3.14. Mean values ± SE of serum GLDH (A) and serum GGT concentrations (B) of the goats infected with 185 (●) or 80 metacercariae (■) and uninfected controls (O).

8.4 Experiment 4: Experimental infection of Nepalese hill buffaloes with high dose (1500) of *F. gigantica* metacercariae

8.4.1 *Clinical data*

Up to 8 weeks after infection there was little to distinguish the infected animals from the controls. Thereafter the infected buffalo calves rapidly developed clinical signs of depression, loss of appetite, dryness of the muzzle and excessive ocular discharge. The first animal to show the signs was calf No. 92, followed by 83, 85 and 89 during the next week. At week 11-12 post infection, marked abdominal distension, weakness, disinclination to move and pallor of the mucous membranes were observed in these calves. Development of similar signs was observed in the remaining infected calves (Nos. 81, 87, 94 and 96) between 12-15 WPI. All the infected calves became progressively anaemic, weaker and listless during the weeks before death or slaughter (Figure 8.4.1). Depraved appetite was observed in calves 81, 87, 92 and 94.

Calf 83 showed a marked dyspnoea and jaundice and died suddenly at 13½ WPI. After the development of sub-mandibular oedema 14 WPI, the condition of calf 92 deteriorated rapidly and it died 4 days later (14½ WPI). This animal had severe diarrhoea for the last 2 days of its life. Similarly, sub-mandibular oedema with rapid deterioration in condition was observed in calves 85 and 89 from 15 WPI. They were slaughtered 17¼ WPI when moribund. Calf 81 also had sub-mandibular oedema (Figure 8.4.1). This calf and calf 94 were unable to stand and move without support before slaughter at 26 and 25¼ WPI respectively. No deviation from the normal state of health was detected in the uninfected controls during the experimental period.

8.4.2 *Liveweight data*

A progressive depression of the mean liveweights of the infected group started from 8 WPI (Figure 8.4.2). The relative increase in the mean liveweight of the infected group after 17 and 26 WPI was due to the death of lighter animals in the group. When compared using the Student's t-test, the mean liveweight of the infected and uninfected groups as the experiment progressed were not significantly different (Appendix Table 8.4.1).

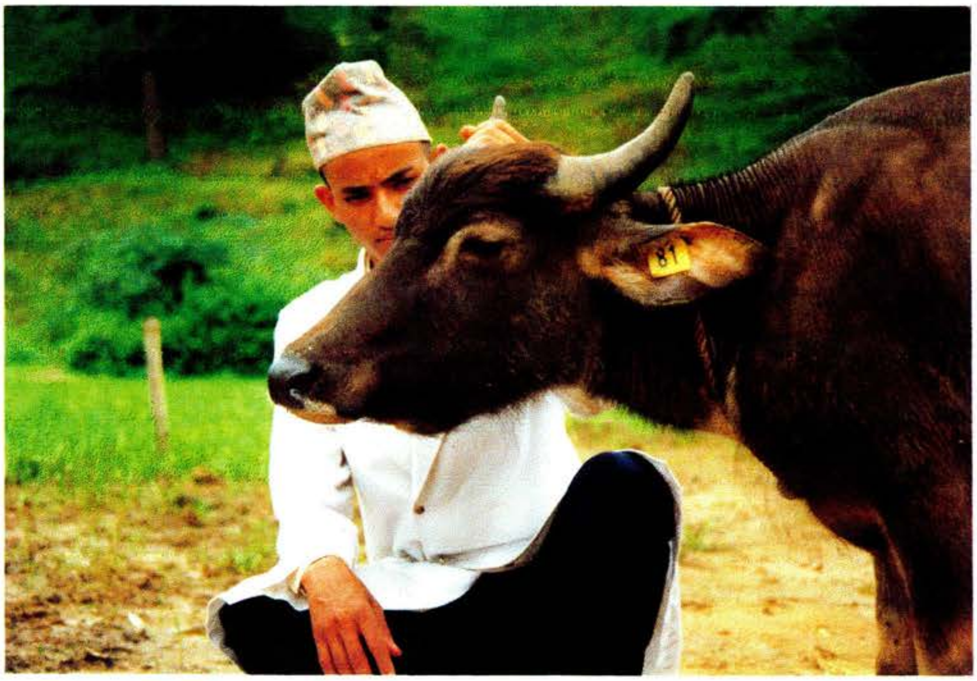


Figure 8.4.1. Top: calf 81 (on right), with a liver fluke burden of 392 *F. gigantica*, 23 weeks after infection; the calf is eating soil. Calf 82, the uninfected pair is on the left. Bottom: calf 81 showing sub-mandibular oedema, 25 weeks after infection.

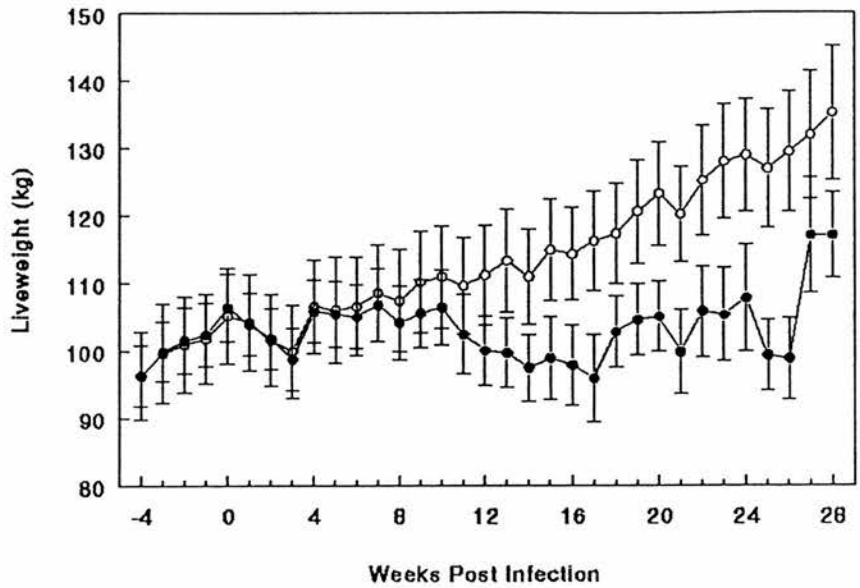


Figure 8.4.2. Mean liveweight ± SE of infected (●) and uninfected (O) buffalo calves in experiment 4.

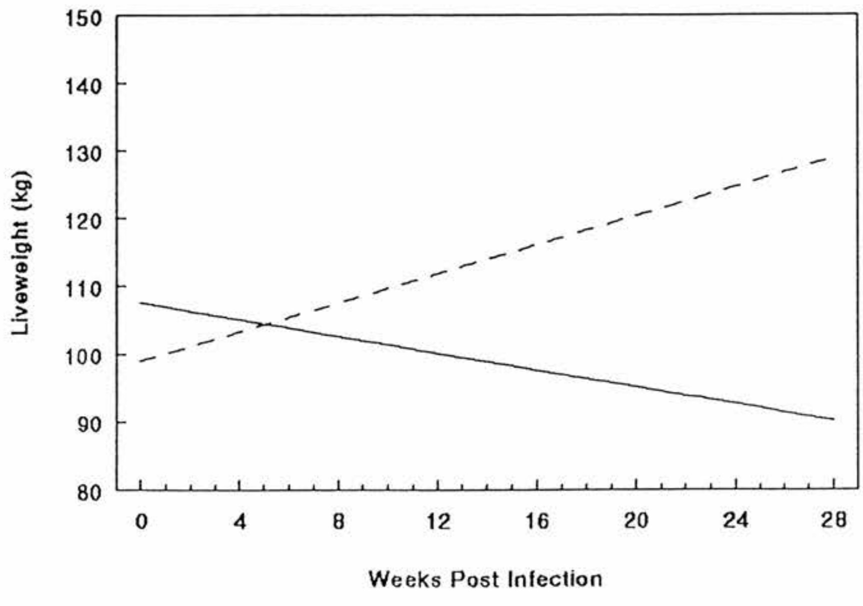


Figure 8.4.3. Lines drawn from the means of the regression coefficients and the Y-axis intercepts of liveweight against weeks post infection for each buffalo calves in the experimental group; infected (—) and uninfected (---) group. This presentation allows for the fact that the lighter animals started to die earlier in the study.

Regression of liveweights against WPI (from 0 WPI to the last weighing) showed that the infected animals lost weight ranging from 0.196 to 1.239 kg/week with an average of 0.618 kg/week, while the uninfected controls gained ranging from 0.337 to 1.580 kg/week with an average of 1.066 kg/week (Appendix Table 8.4.2). The liveweight gains (i.e. slopes of the regression lines) of the matched pairs from infected and uninfected groups were significantly different ($F = > 27.00$, d.f. = 1, $P = < 0.001$) (Appendix Table 8.4.3). The mean slopes of the regression lines of infected and uninfected groups (Figure 8.4.3) were also significantly different ($F = 62.89$, d.f. = 14, $P = < 0.001$).

The liveweight loss of the infected buffaloes significantly correlated to fluke burdens (Figure 8.4.4). The regression indicated that each fluke reduced liveweight of the infected buffaloes by about 2.6 g/per week.

8.4.3 *Parasitological data*

The raw data for *F. gigantica* faecal egg counts is presented in Appendix Table 8.4.4. The pre-patent period for these *F. gigantica* infections varied from 90 and 104 days with an average of 97 days (Table 8.4.1). Regression analysis showed that the course of egg production increased in an exponential fashion (Figure 8.4.5). The recovery of *F. gigantica* at slaughter/death of the buffaloes ranged from 25.5 to 56.5% with a mean value of 40% (Table 8.4.1).

A few (100-150 EPG) strongyle-type eggs were seen around 22 WPI in almost all the buffaloes. On occasions, small number of paramphistome eggs in calf 81 and *Eurytrema* spp. eggs in calves 84, 87 and 94 were also detected.

A total of 13 paramphistomes were recovered from the rumen of calf 81. Examination of the pancreatic ducts revealed the presence of 21, 38 and 130 mature *Eurytrema cladorchis* in calves 84, 87 and 94 respectively. Adult *Haemonchus* spp. were recovered from the abomasum of most of the animals but not more than 10 worms from any one animal.

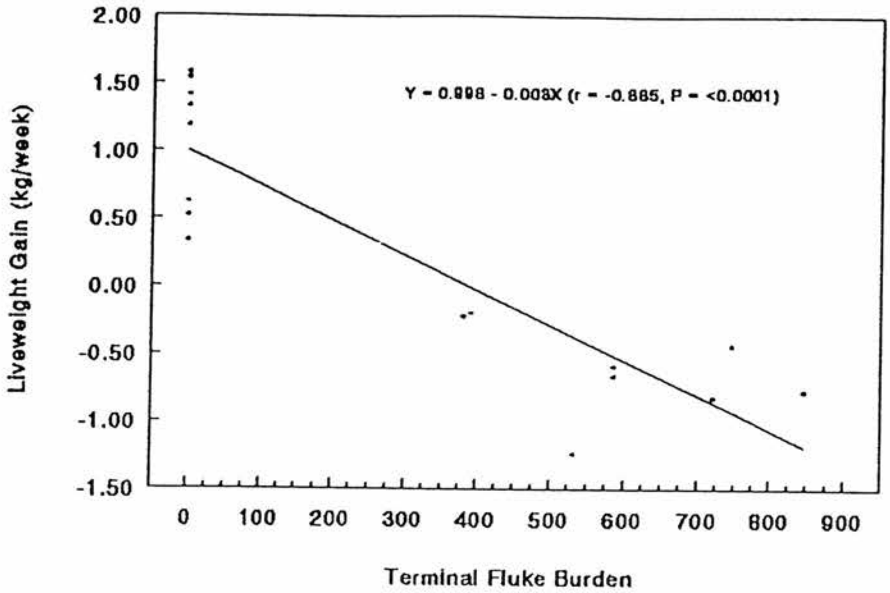


Figure 8.4.4. Regression line showing the relationship between the fluke burden and liveweight gain in buffaloes in experiment 4.

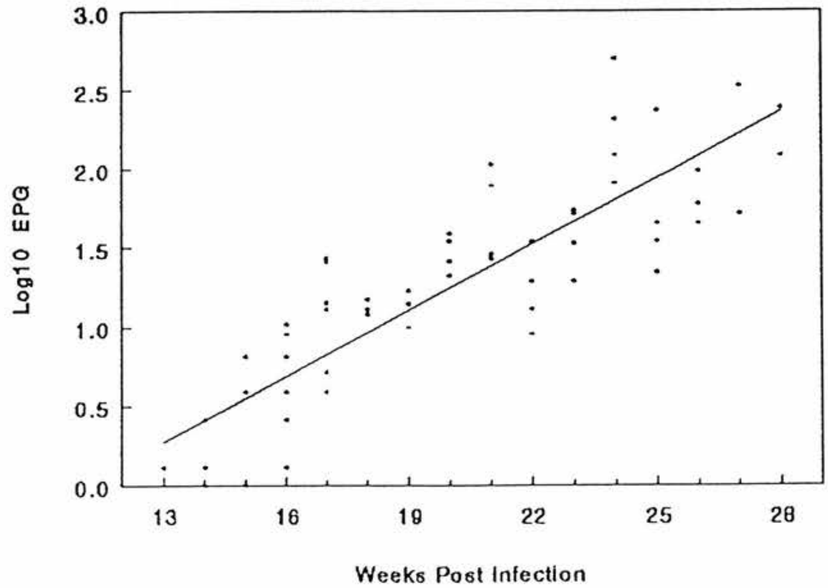


Figure 8.4.5. Regression line (Y = -1.537+0.139X, r^2 = 0.75, P < 0.001) for the scatter plot of *Fasciola gigantica* faecal egg counts ($\log_{10}$ EPG) of the infected buffalo calves in experiment 4.

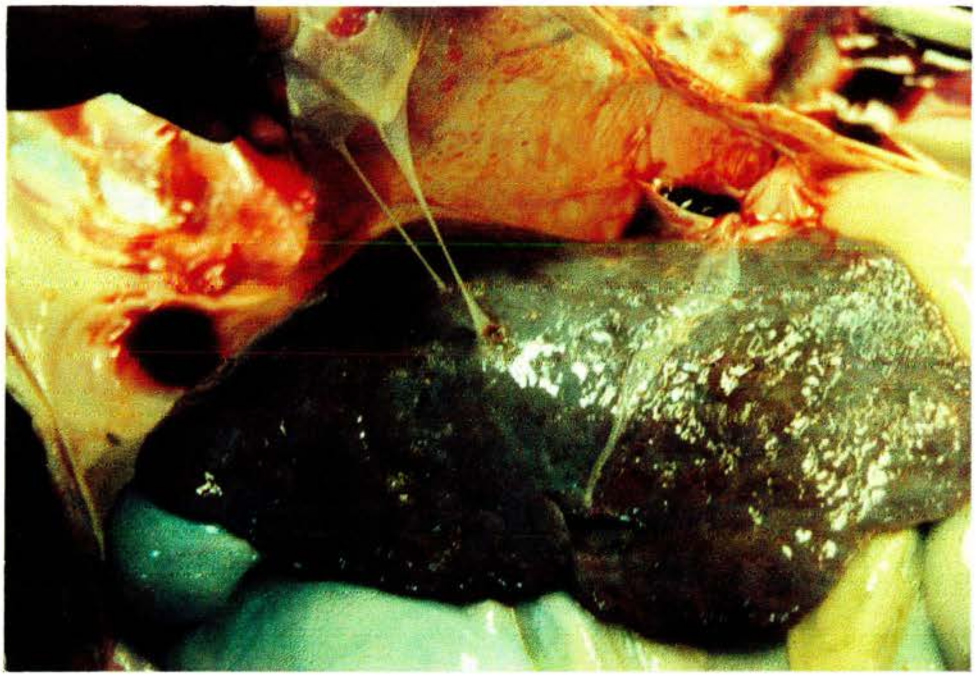
Table 8.4.1. Some parasitological details of the buffalo calves of infected group in experiment 4.

Calf No.	Pre-patent period (days)	Days (weeks) from infection to death	Fluke recovery		Mean (± SD) fluke size (mm)	
			No.	%	Length	Width
81	99	183 (26)	392	26.1	34.1 (± 4.0)	6.7 (± 0.9)
83	95	95 (13½)	587	39.1	23.1 (± 3.0)	3.9 (± 0.9)
85	96	121 (17½)	747	49.8	30.8 (± 3.4)	6.0 (± 0.9)
87	98	195 (28)	587	39.1	36.5 (± 4.6)	6.6 (± 0.9)
89	104	121 (17½)	847	56.5	27.2 (± 4.0)	4.9 (± 0.7)
92	90	101 (14½)	721	48.1	28.0 (± 5.0)	5.5 (± 1.3)
94	98	177 (25¼)	533	35.5	38.0 (± 4.9)	7.9 (± 0.9)
96	97	195 (28)	382	25.5	38.8 (± 4.2)	7.0 (± 1.1)
Mean	97	149 (21¼)	600	40.0	32.1	6.1

8.4.4 *Pathological data*

The carcasses of the infected buffaloes were cachectic. The peritoneal cavities contained large quantities of fluid; the fluid was serosanguinous in the calves which died within 15 WPI (Nos. 83 and 92). All the visceral organs were very pale and there was serous atrophy of mesenteric fat particularly in calves 81, 87, 94 and 96. In most cases, however, specific lesions were confined to the liver.

The livers of infected buffalo calves were enlarged with thickened capsules, while the hepatic lymph nodes were grossly enlarged as were the gall bladders which were also black in colour. The mean liver weight of the infected buffaloes was significantly heavier than that of the uninfected group (Appendix Table 8.4.4a). The livers of calves 83, 85, 89 and 92 had a mosaic appearance of greyish and haemorrhagic areas 1 to 4 mm in diameter. There were scattered areas of fibrinous adhesion to the abdominal wall and diaphragm (Figure 8.4.6). On the liver surfaces there were perforations of 1 to 3 mm diameter, through which protruded live flukes. Many flukes were found on the surface of liver. The cut surfaces of the livers showed numerous flukes in distended bile ducts and in the liver parenchyma. The fluke tracts were haemorrhagic in many places especially around the bile ducts (Figure 8.4.6). The livers of calves 81, 87, 94 and 96 were cirrhotic. The main bile ducts were markedly dilated and prominent. The walls of the bile ducts were thickened and fibrotic but not calcified.



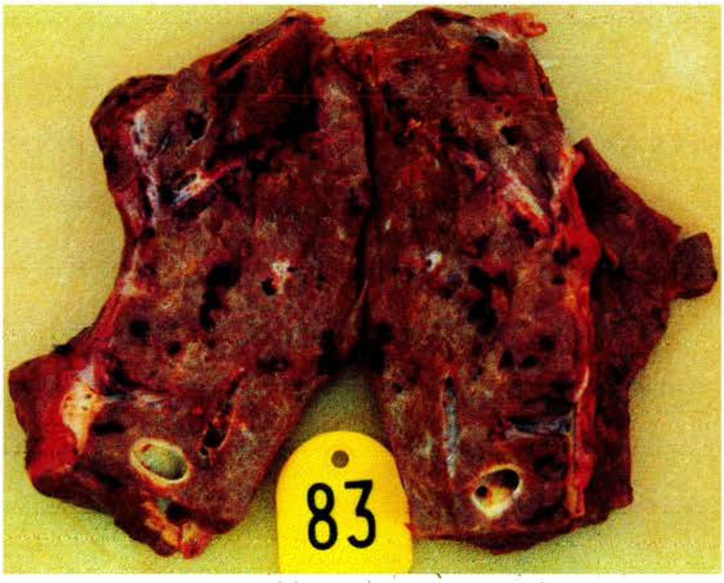


Figure 8.4.6. Top: liver of calf 89 *in situ* with a burden of 847 *F. gigantica* and slaughtered 17¼ WPI, showing surface lesions and fibrinous adhesions to the diaphragm. Bottom: liver of calf 83 with a liver fluke burden of 587 *F. gigantica* which died 13½ WPI, showing haemorrhagic tracts in the parenchyma.

Calves 83, 85, 89 and 92 also had lung lesions. There were greenish grey raised nodules about 2-3 cm in diameter on the surface. Incision of the lesions revealed cavities filled with greenish grey turbid fluid containing live flukes.

Lesions were widespread in calf 83. The carcass was severely jaundiced. There were petechial and diffuse haemorrhages in subcutaneous tissue; blood stained fluid in the peritoneal, pleural and pericardial cavities; petechial haemorrhages under the endocardium and in the kidneys; haemorrhagic enteritis accompanied by the presence of blood and blood stained ingesta and anaemic infarcts in the liver. The urine was red and the gall bladder was filled with a blood clot. Several large blood clots were present in the peritoneal cavity. Ectopic flukes were recovered from the haemorrhagic nodules in the intestine and oesophagus.

Histopathological examination of sections of liver tissue showed widespread periportal, and some perilobular fibrosis. In most areas the fibrous tissue was relatively immature and cellular. Periportal areas were infiltrated by low to moderate numbers of mononuclear inflammatory cells (plasma cells, lymphocytes and monocytes). Bile ducts in portal zones exhibited mild proliferative changes (Figure 8.4.7). The epithelium of gall bladders and major bile ducts was ulcerated in a few areas and associated with haemorrhage and necrosis (Figure 8.4.8). Mucous crypts and glands were quite prominent with some Goblet cell hyperplasia. A thick band of fibrous tissue separated the duct and the hepatic parenchyma. No calcification was noted in the ducts. Hepatocytes in most areas were either unremarkable or showed mild degenerative changes (cytoplasmic swelling and vacuolation)(Figure 8.4.8). In a few focal areas necrotic lesions of varying size were present with degenerate or absent hepatocytes, haemorrhage and many neutrophil polymorphs. Adjacent areas were often markedly fibrosed with very disrupted parenchymal architecture (Figure 8.4.9). Necrotic and fibrosis lesions in calf 83 were particularly marked and in some areas little unaffected parenchyma survived. Flukes were evident within some necrotic and inflammatory lesions in the liver (Figure 8.4.9). Other tissues from calf 83 examined and demonstrated haemorrhagic foci in the urinary bladder submucosa, in the subendocardium and pericardium, and in the duodenal submucosa.

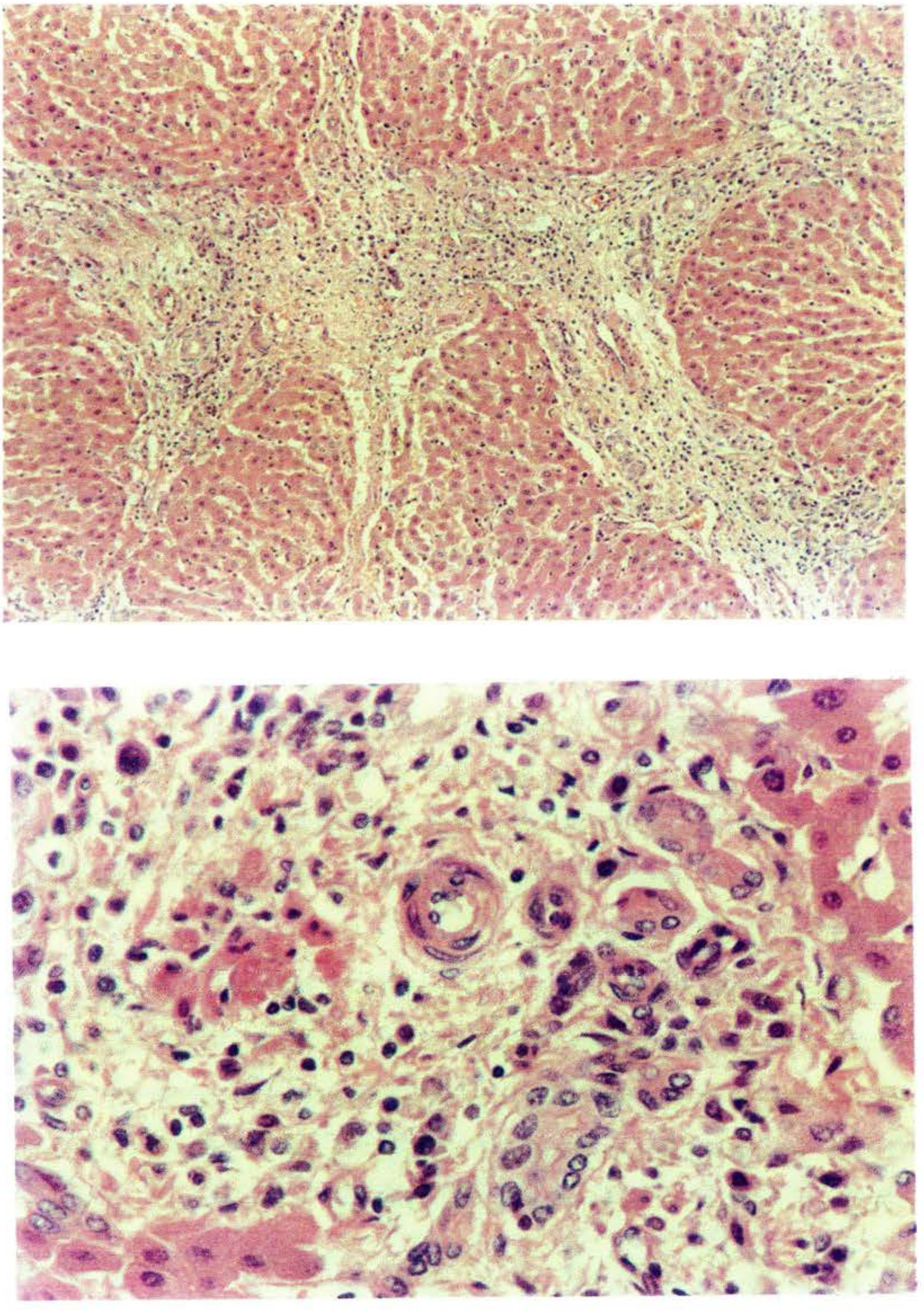


***Figure** 8.4.7.* Photomicrographs of the section of liver of buffalo 85: top plate showing general periportal fibrosis (H&E stained, x130 magnification); bottom plate showing bile duct proliferation and mononuclear inflammatory cells infiltration (H&E stained, x520 magnification).

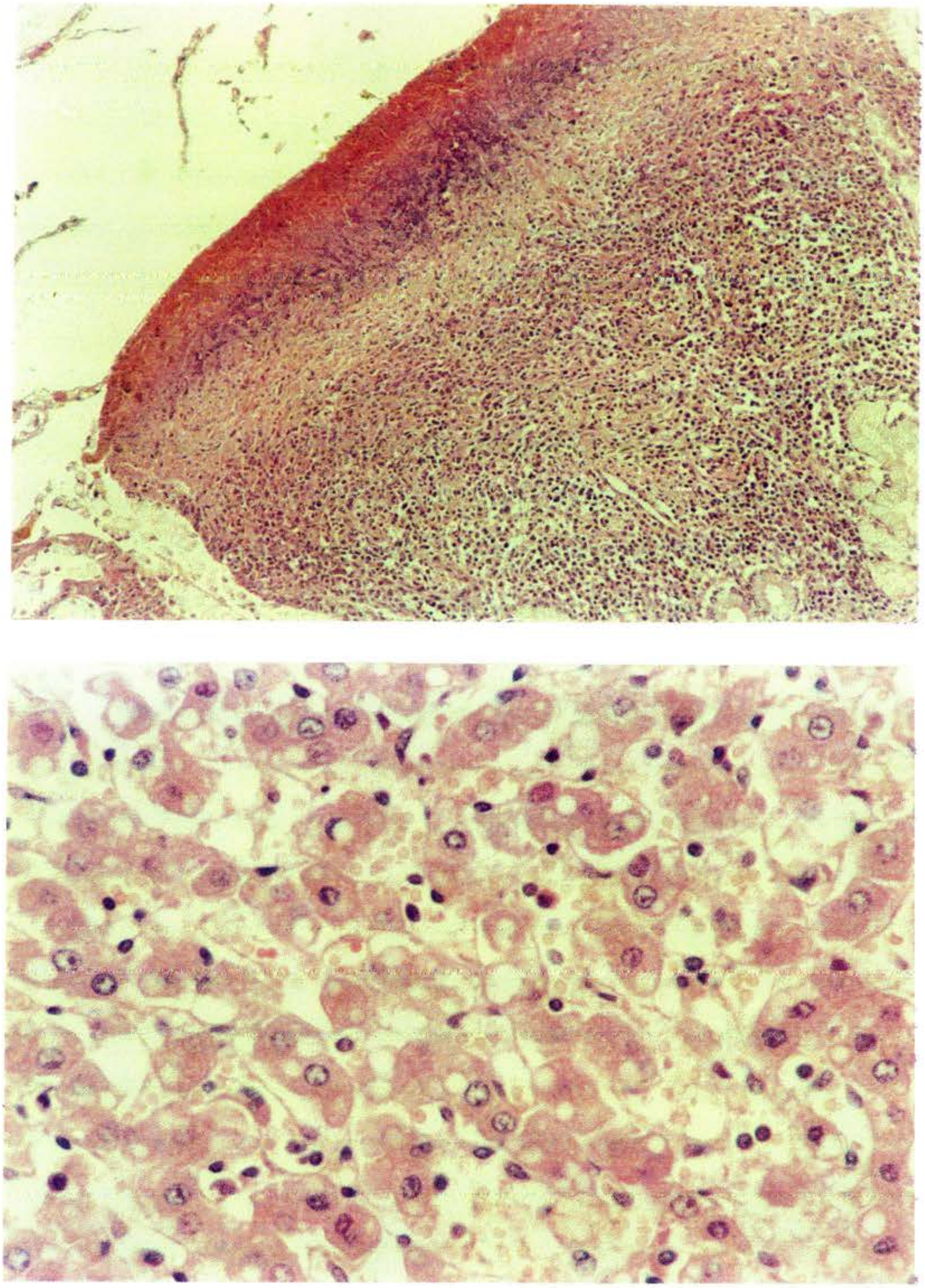


Figure 8.4.8. Photomicrographs of the section of liver of buffalo 94: top plate showing ulceration and necrosis of gall bladder epithelium with infiltration of many mononuclear inflammatory cells (H&E stained, x52 magnification); bottom plate showing hepatocyte degeneration with marked cytoplasmic swelling and vacuolation (H&E stained, x520 magnification).

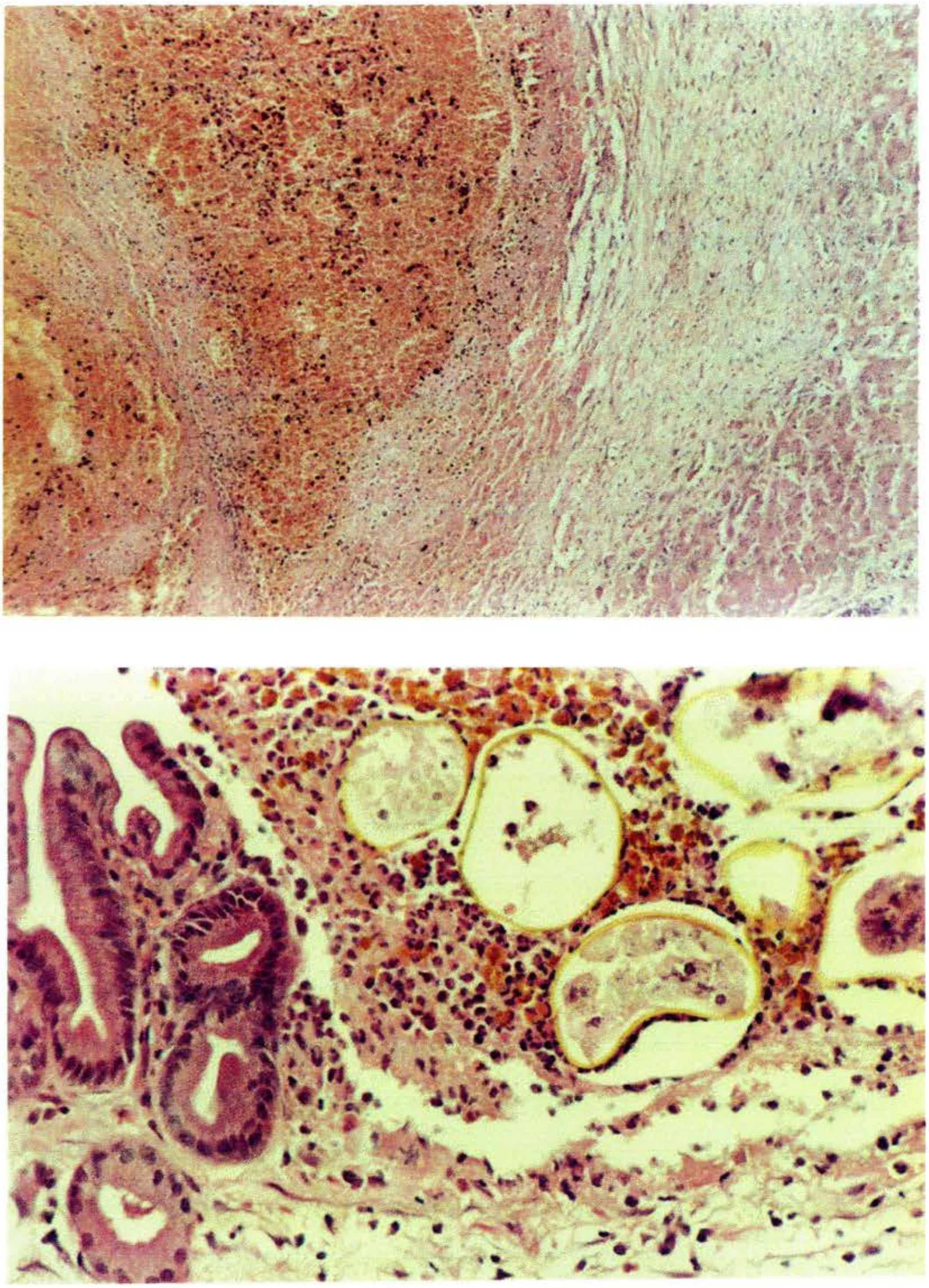


Figure 8.4.9. Photomicrographs of the section of liver of buffalo 83: top plate showing marked parenchymal fibrosis and necrosis associated with haemorrhage (H&E stained, x52 magnification); bottom plate showing immature flukes in necrotic and inflammatory lesions (H&E stained, x260 magnification).

Clinical and pathological findings suggested that the death of calf 83 was either due to black disease or bacillary haemoglobinuria associated with the liver lesions caused by the immature migratory flukes. Therefore samples of the heart blood and liver tissues were processed for bacteriological examinations. The organisms isolated from anaerobic culture on blood agar were tentatively identified as *Clostridium* spp. on the basis of cultural and morphological characteristics. The isolate was further identified as *Cl. novyi* by direct fluorescent antibody technique. Biochemical tests confirmed that the isolate belonged to *Cl. novyi* type D (*Cl. haemolyticum*), the causative organism of bacillary haemoglobinuria.

8.4.5 *Haematological data*

The infected buffaloes showed a progressive drop in the mean RBC counts, PCVs and haemoglobin concentrations beginning 2 to 5 weeks after infection, while the uninfected group maintained its original level (Figure 8.4.10). The Mann-Whitney test demonstrated that the RBC counts, PCVs and haemoglobin concentrations of the infected and uninfected groups differed significantly from 13, 10 and 12 WPI respectively (Appendix Tables 8.4.5, 8.4.6 and 8.4.7). Regression analysis showed that these falls in the infected buffaloes followed a linear pattern (Table 8.4.2). The rates of decline of all these parameters appeared to be positively correlated with fluke burdens. The highest correlation coefficient was obtained for haemoglobin ($r = 0.9388$, d.f. = 6, $P = 0.0005$) followed by PCV ($r = 0.7273$, d.f. = 6, $P = 0.0409$) and RBC ($r = 0.6730$, d.f. = 6, $P = 0.0674$). When the data for calf 92 was omitted from the analyses, all the correlation coefficients increased and the correlation between rate of decline in RBC counts and fluke burdens also became significant ($r = 0.7988$, d.f. = 5, $P = 0.0312$).

The mean MCV, MCH and MCHC values for the two groups are illustrated in Figure 8.4.11. The mean MCV of the infected buffaloes increased from week 16 PI, and the differences with those of the uninfected controls became significant from week 21 PI (Appendix Table 8.4.8.). There were no significant changes in the values of MCH (Appendix Table 8.4.9), but a significant decline in the MCHC of the infected group was observed from week 13 post infection (Appendix Table 8.4.10).

Table 8.4.2. Regression statistics of RBC counts, PCVs and haemoglobin concentrations of the infected buffalo calves against WPI (0 WPI to last sampling) in experiment 4.

Calf No	n	RBC counts			Packed cell volumes			Haemoglobin concentrations		
		Equations	r^2	P	Equations	r^2	P	Equations	r^2	P
81	27	Y = 6.82-0.17X	0.80	< 0.0001	Y = 0.33-0.008X	0.88	< 0.0001	Y = 129.5-3.76X	0.83	< 0.0001
83	15	Y = 8.64-0.28X	0.50	0.0033	Y = 0.38-0.013X	0.60	0.0007	Y = 143.9-4.24X	0.44	0.0066
85	19	Y = 7.96-0.31X	0.87	< 0.0001	Y = 0.36-0.013X	0.86	< 0.0001	Y = 134.2-5.00X	0.66	< 0.0001
87	29	Y = 7.80-0.19X	0.89	< 0.0001	Y = 0.35-0.007X	0.94	< 0.0001	Y = 135.4-3.24X	0.85	< 0.0001
89	19	Y = 6.85-0.28X	0.86	< 0.0001	Y = 0.36-0.014X	0.93	< 0.0001	Y = 141.2-5.61X	0.87	< 0.0001
92	16	Y = 5.39-0.16X	0.70	< 0.0001	Y = 0.29-0.009X	0.85	< 0.0001	Y = 118.9-4.18X	0.78	< 0.0001
94	27	Y = 6.25-0.16X	0.85	< 0.0001	Y = 0.30-0.006X	0.90	< 0.0001	Y = 119.8-2.60X	0.84	< 0.0001
96	29	Y = 6.19-0.11X	0.74	< 0.0001	Y = 0.32-0.004X	0.56	< 0.0001	Y = 128.6-2.09X	0.78	< 0.0001

The total WBC counts of the infected group were relatively higher during 4 to 12 WPI and lower during 21 to 26 WPI than the controls (Figure 8.4.12), however, the differences were not significant (Appendix Table 8.4.11). Eosinophil counts in the infected buffaloes rose by the 2nd week after infection and reached a peak shortly after that at week 3 PI (Figure 8.4.12). A second peak occurred at 13 WPI. Statistically, the eosinophil counts of the infected and uninfected groups were significantly different from week 2 to week 13 PI (Appendix Table 8.4.12). The correlation analyses showed that there were no associations between mean eosinophil counts and fluke burdens during 2 to 7 ($r = 0.0340$, d.f. = 6, $P = 0.9363$), 8 to 20 ($r = -0.2803$, d.f. = 6, $P = 0.5013$) or 2 to 13 WPI ($r = 0.0548$, d.f. = 6, $P = 0.8974$).

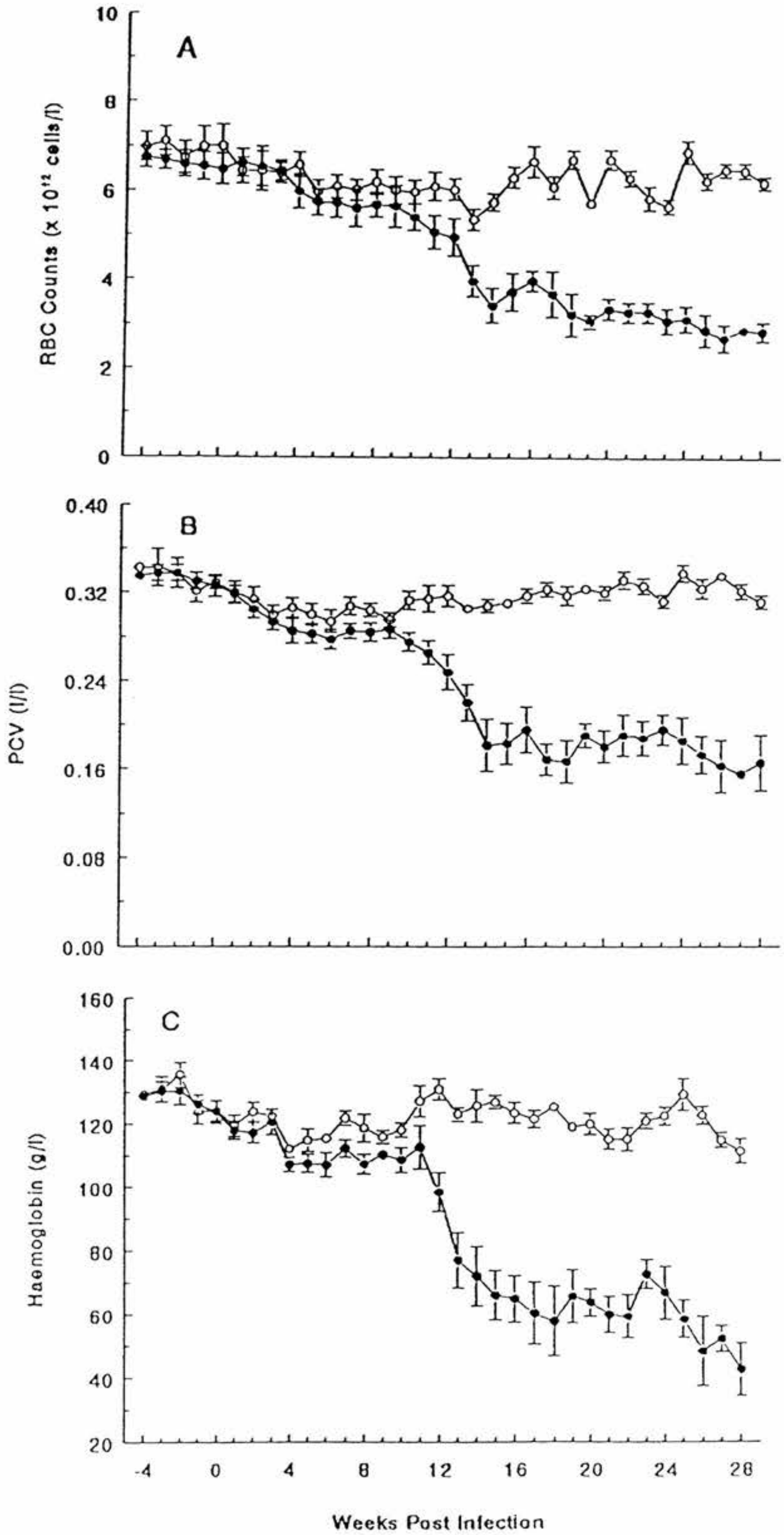


Figure 8.4.10. Mean values ± SE of RBC counts (A), PCV (B) and haemoglobin concentrations (C) of the infected (●) and uninfected (O) groups of buffalo calves in experiment 4.

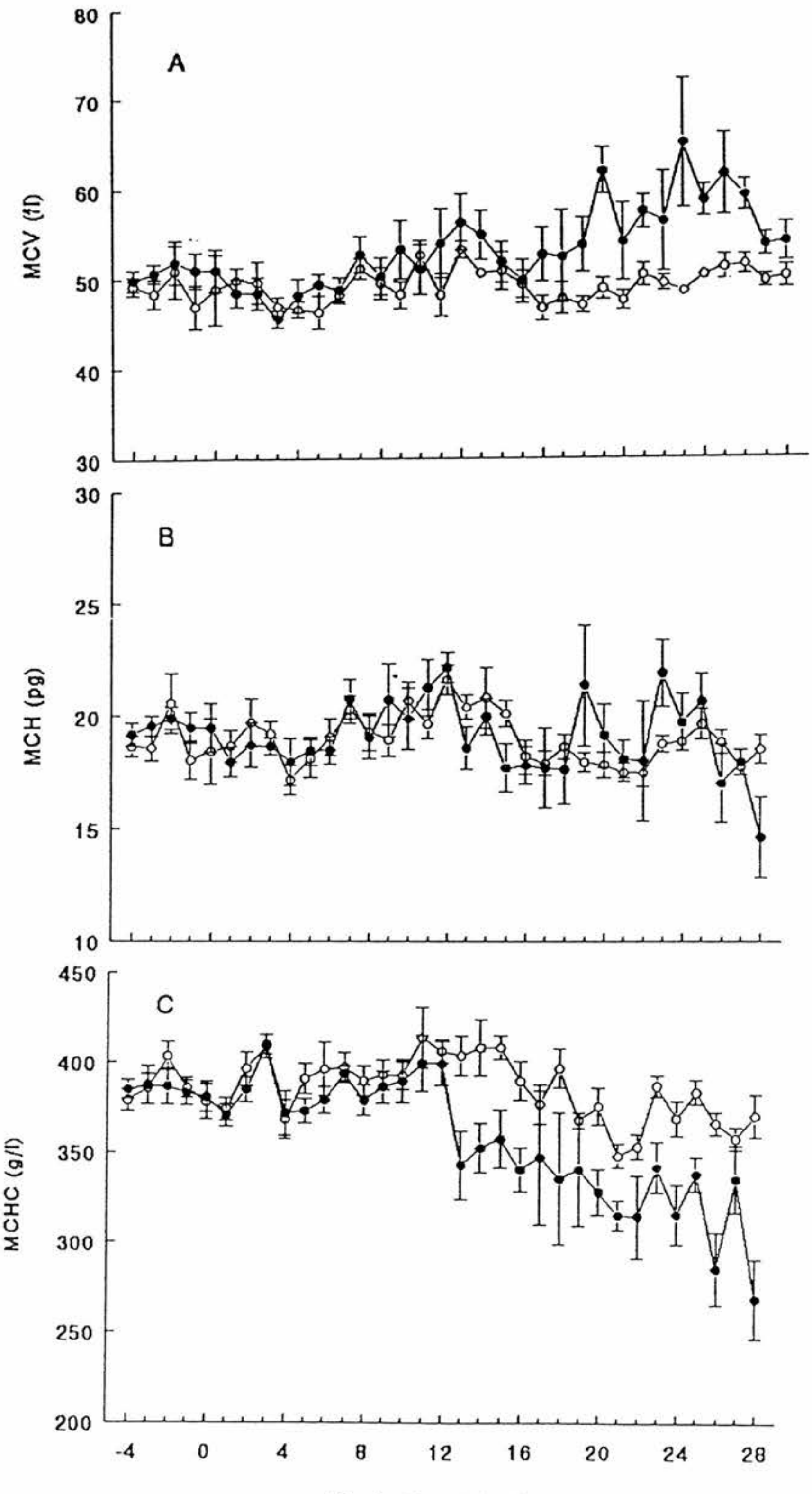


Figure 8.4.11. Mean values ± SE of MCV (A), MCH (B) and MCHC (C) of the infected (●) and uninfected (O) groups of buffalo calves in experiment 4.

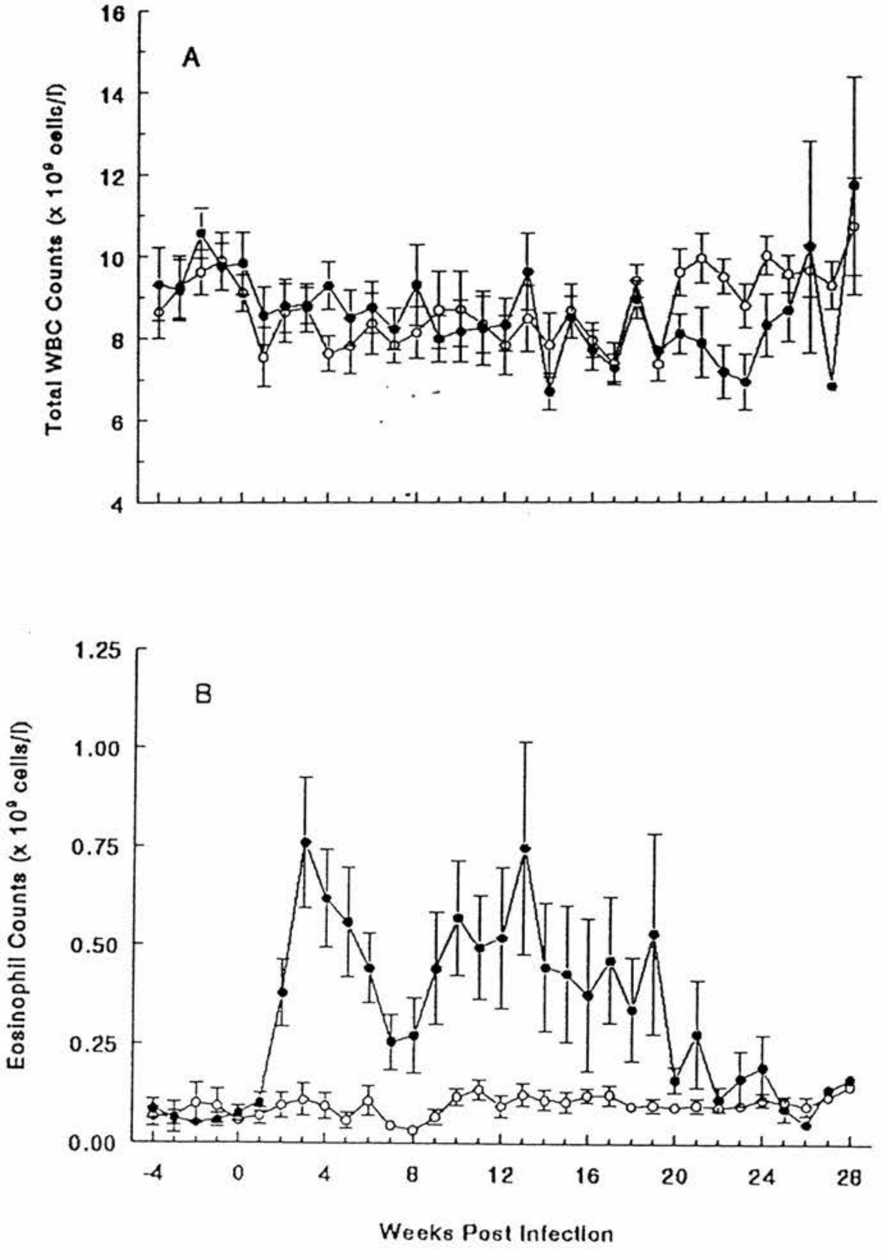


Figure 8.4.12. Mean values $\pm$ SE of total WBC (A) and eosinophil (B) counts of the infected (●) and uninfected (O) groups of buffalo calves in experiment 4.

8.4.6 Biochemical data

A slight rise in total serum protein concentration of the infected buffaloes was noticed 4 weeks after infection and this remained high until 12 WPI (Figure 8.4.13). The levels of infected and uninfected groups differed significantly at 12, 13 and 14 WPI (Appendix Table 8.4.13). Thereafter, the serum protein concentrations in the infected buffaloes declined progressively (except for calf 96 in which the onset of the decline was delayed until 15 WPI), but the differences with those of the uninfected group were not significant. Regression analysis showed that both the rises until 12 WPI and falls thereafter in protein concentrations of the individual animals (except for calves 89 and 96 for the elevated levels) were in linear fashions (Table 8.4.2). Although there appeared to be a positive correlation between the initial rise in total serum protein concentration and fluke burden, this was not significant (r = 0.5410, d.f. = 6, P = 0.1661). However, there was a significant positive correlation between the rate of decline and fluke burden found at post mortem (r = 0.8961, d.f. = 4, P = 0.0156).

Table 8.4.2. Regression statistics of total serum protein levels of the infected buffalo calves against WPI in experiment 4.

Calf No.	Rising levels (from 0 to 12 WPI)				Declining levels (from 13 WPI to last sampling)			
	Equations	n	r^2	P	Equations	n	r^2	P
81	Y = 68.19+0.58X	13	0.47	< 0.001	Y = 109.87-2.90X	14	0.96	< 0.001
83	Y = 62.65+0.58X	13	0.30	0.042	NA	NA	NA	NA
85	Y = 62.61+0.95X	13	0.76	< 0.001	Y = 128.12-4.51X	6	0.86	0.008
87	Y = 61.17+0.79X	13	0.56	0.003	Y = 93.97-2.05X	16	0.82	< 0.001
89	Y = 63.65+0.64X	13	0.29	0.059	Y = 141.28-5.31X	6	0.85	0.009
92	Y = 51.78+1.12X	13	0.61	0.001	NA	NA	NA	NA
94	Y = 59.39+0.86X	11	0.63	< 0.001	Y = 128.32-3.68X	11	0.96	< 0.001
96	Y = 67.16+0.45X	16	0.17	0.106	Y = 99.54-1.68X	13	0.85	< 0.001

NA = Sufficient data not available for analysis because of the early deaths.

Against a background of week to week variation until 12 WPI, the levels of serum albumin concentrations in the infected group declined significantly 13 weeks after infection (Appendix Table 8.4.14 and Figure 8.4.13). The decline in concentrations thereafter was linear. However, this was not the case for calves 83 and

92 which died before 15 WPI (Table 8.4.3). There was a positive correlation between the rate of decline in serum albumin concentrations and the terminal fluke burdens (r = 0.8612, d.f. = 4, P = 0.0276).

Table 8.4.3. Regression statistics of serum albumin concentrations of the infected calves against WPI (from 12 WPI to the last sampling) in experiment 4.

Calf No.	Equations (Y = a+b.X)	n	r^2	P
81	Y = 40.73-1.06X	14	0.65	0.0005
83	Y = 94.15-5.15X	3	0.83	0.2713
85	Y = 60.42-2.76X	7	0.78	0.0085
87	Y = 38.90-0.85X	17	0.58	0.0004
89	Y = 55.03-2.31X	7	0.59	0.0443
92	Y = 110.43-6.25X	3	0.85	0.2533
94	Y = 39.71-1.00X	15	0.65	0.0003
96	Y = 30.49-0.50X	17	0.22	0.0486

The calculated values together with the results of statistical comparisons between infected and uninfected buffaloes for serum globulin and serum albumin to serum globulin ratios are presented in Appendix Tables 8.4.15 and 8.4.16 respectively. The serum globulin concentrations in the infected buffaloes increased from 4 WPI to reach the peak level at 15 WPI (Figure 8.4.14). The higher level of serum globulin in the infected buffaloes differed significantly from those of the uninfected buffaloes from 13 to 17 WPI. After 22 WPI, the levels dropped below those of the uninfected controls, but the differences were not significant. The albumin to globulin ratios appeared to decrease soon after infection, but the differences with those of the uninfected group became significant by 13 WPI.

The results of serum GLDH and serum GGT assays with statistical analyses are given in Appendix Tables 8.4.17 and 8.4.18 respectively and the mean changes are illustrated in Figure 8.4.15. Analysis of the data revealed that the serum GLDH values of infected calves were significantly elevated as early as 2 WPI, reaching a peak of 265.8 U/l at 11 WPI and remaining significantly higher than control values throughout the experimental period. Although there appeared to be a positive correlation between serum GLDH levels (from 2-14 WPI) and terminal fluke burdens, this was not significant (r = 0.1488, d.f. = 6, P = 0.7251).

The serum GGT levels increased from 7 WPI and reached maximum levels in infected buffaloes 12-16 WPI. These levels in the infected group differed significantly from those of the uninfected group from 8 WPI and remained significantly different until the end of experiment. There was no correlation between number of flukes recovered postmortem from the individual calves and the mean GGT activity in the serum from 10 to 16 WPI ($r = 0.2169$, d.f. = 6, $P = 0.6059$).

The results of serum calcium, serum potassium, serum sodium, serum magnesium, serum inorganic phosphorus and serum copper determinations and their statistical analysis are shown in Appendix Table 8.4.19. The group mean values of these serum constituents are presented in Figures 8.4.16 and 8.4.17. These determinations were carried out at monthly as opposed to weekly intervals.

There was a general trend that serum calcium, potassium, sodium, magnesium and inorganic phosphorus concentrations were less in the infected buffaloes than the uninfected controls after 8-12 WPI. Although there appeared declines in serum copper levels in the infected buffaloes from 8 WPI, interpretation was complicated by the erratic trend in the levels in the uninfected controls. Significant differences between the mean values of the two groups were detected for calcium at 20 WPI, potassium at 16 WPI (which appeared to be attributed to rise in level in the uninfected controls), magnesium at 28 WPI and inorganic phosphorus from 20 WPI till end of the experiment (Appendix Table 8.4.19).

8.4.7 *Agar gel diffusion assay*

Sera from most of the infected calves showed single precipitin lines as early as 2 to 3 WPI except for those from calves 89 and 92 in which precipitin reactions were detected 7 and 12 WPI respectively. As the experiment progressed, 2-3 lines were seen occasionally, however, the lines became fainter and some times absent after 20 WPI.

Sera from all the uninfected control animals gave negative test results during the whole experimental period except for one occasion during pre-infection period (-4 WPI) when a faint precipitin line was observed in the serum from calf 93. Serum from calf 96 (infected) at -4 WPI also showed a faint precipitin line. The raw data of agar gel diffusion tests are presented in Appendix Table 8.4.20.

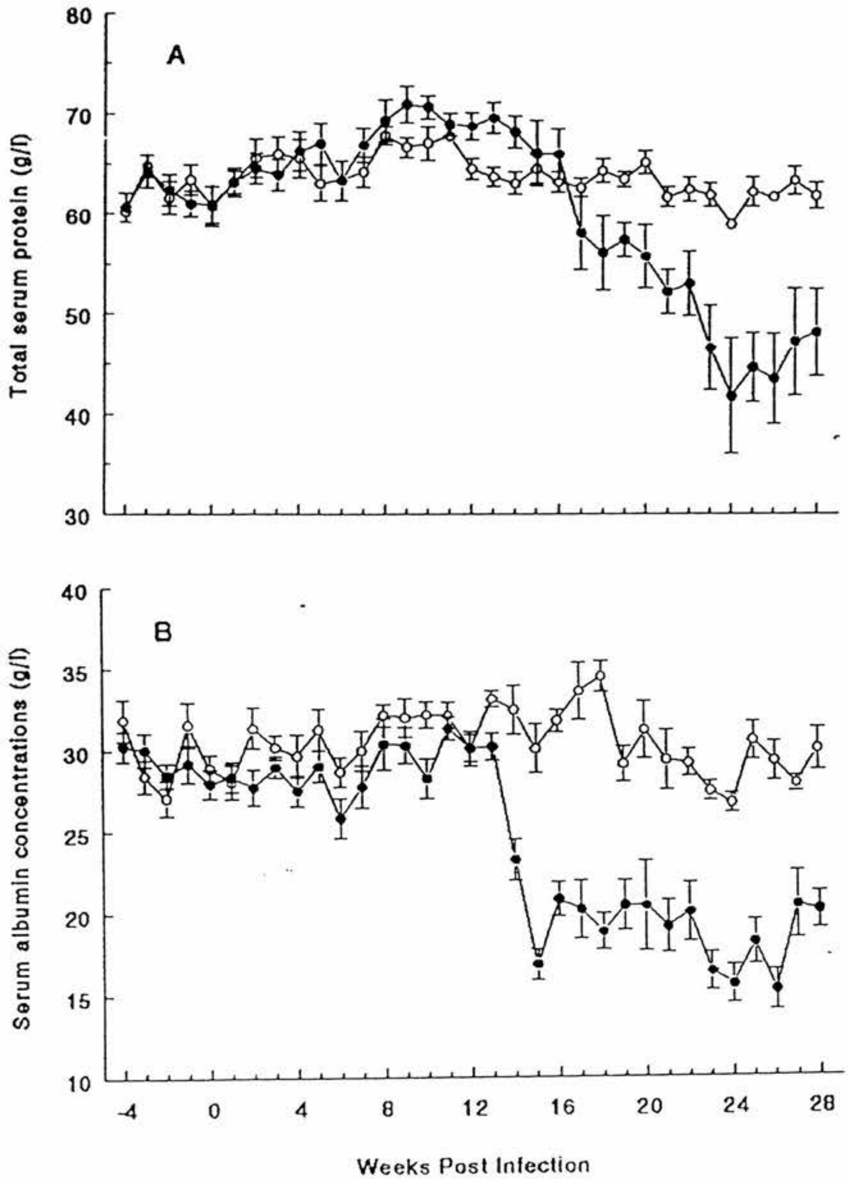


Figure 8.4.13. Mean values ± SE of serum total protein (A) and serum albumin (B) concentrations of the infected (●) and uninfected (O) groups of buffalo calves in experiment 4.

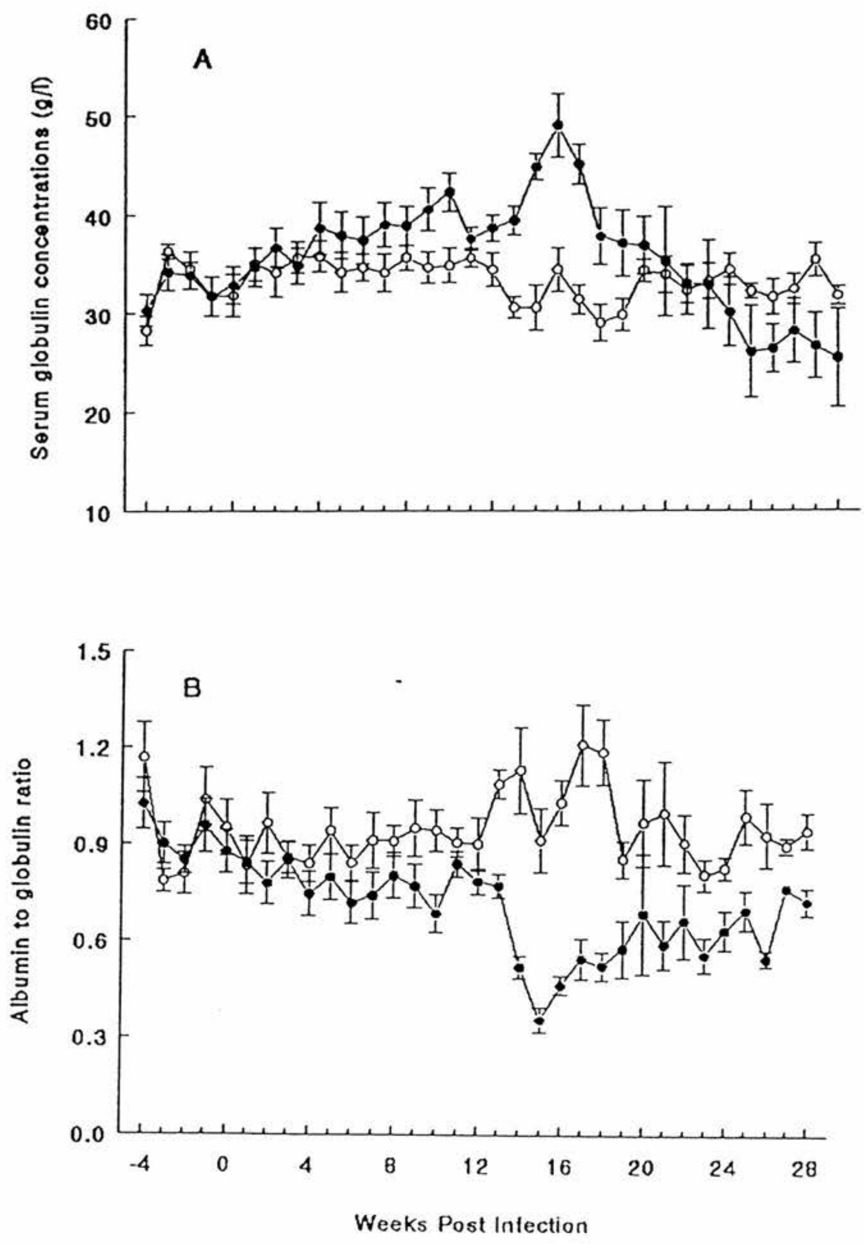


Figure 8.4.14. Mean values ± SE of serum globulin concentrations (A) and serum albumin to serum globulin ratios (B) of the infected (●) and uninfected (O) groups of buffalo calves in experiment 4.

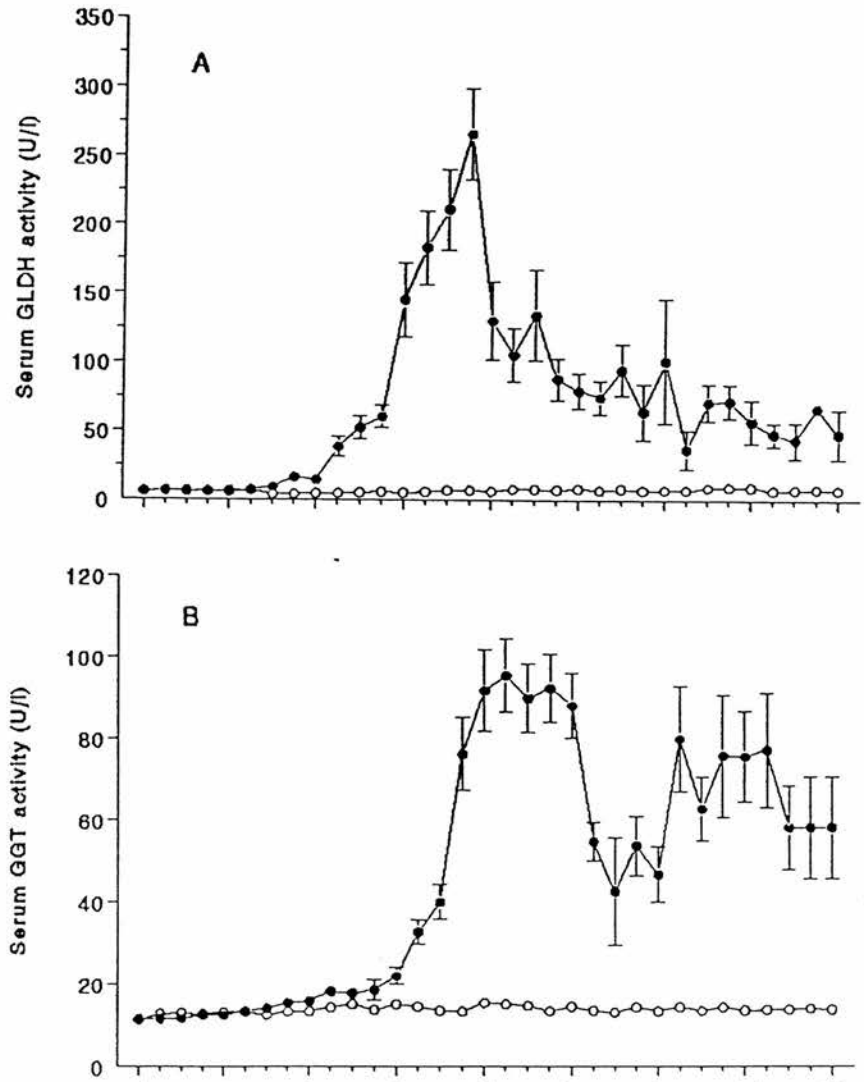


Figure 8.4.15. Mean values ± SE of the levels of GLDH (A) and GGT (B) in the sera of infected (●) and uninfected (O) groups of buffalo calves in experiment 4.

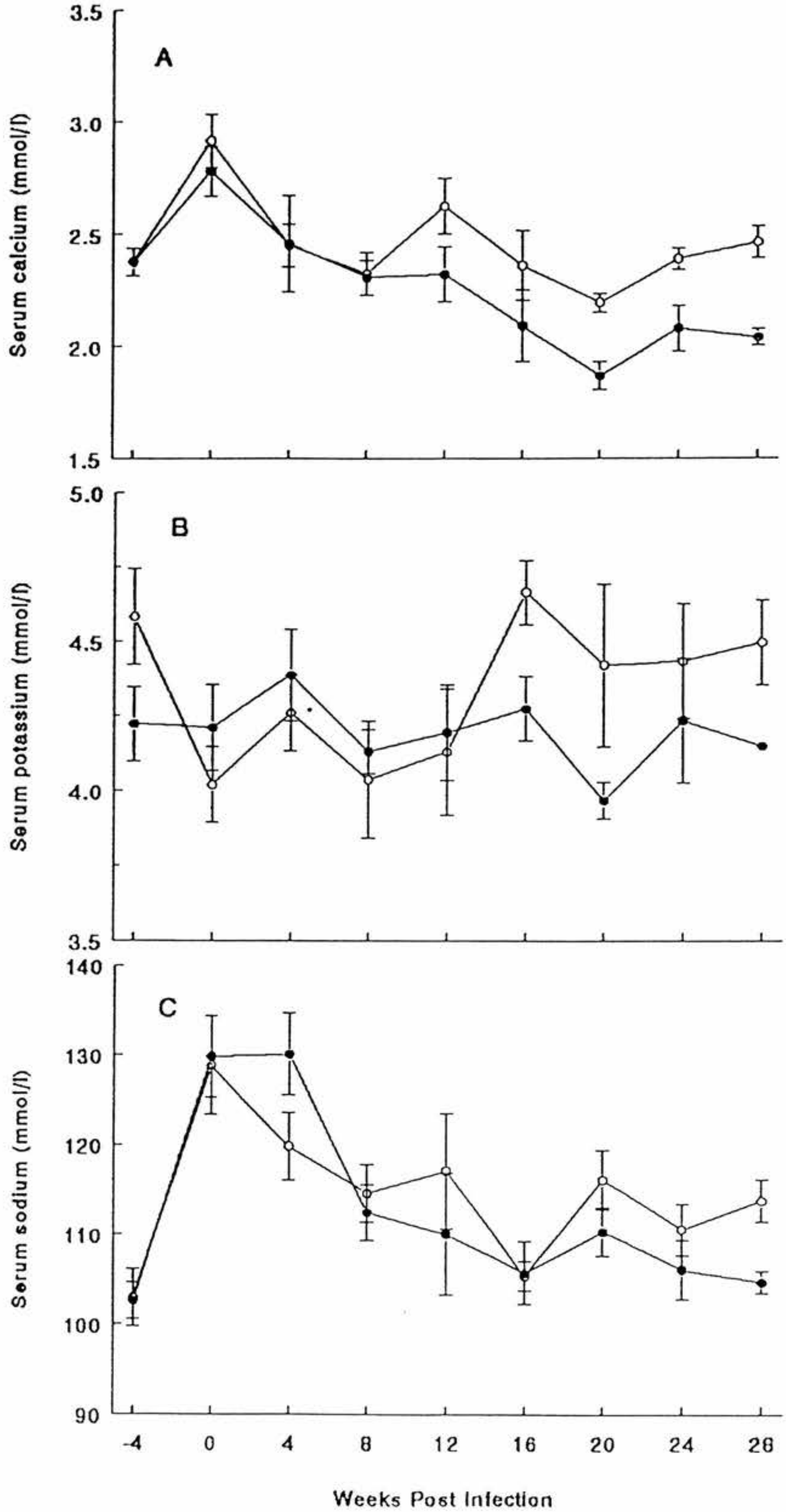


Figure 8.4.16. Mean values ± SE of serum calcium (A), serum potassium (B) and serum sodium (C) concentrations of the infected (●) and uninfected (O)groups of buffalo calves in experiment 4.

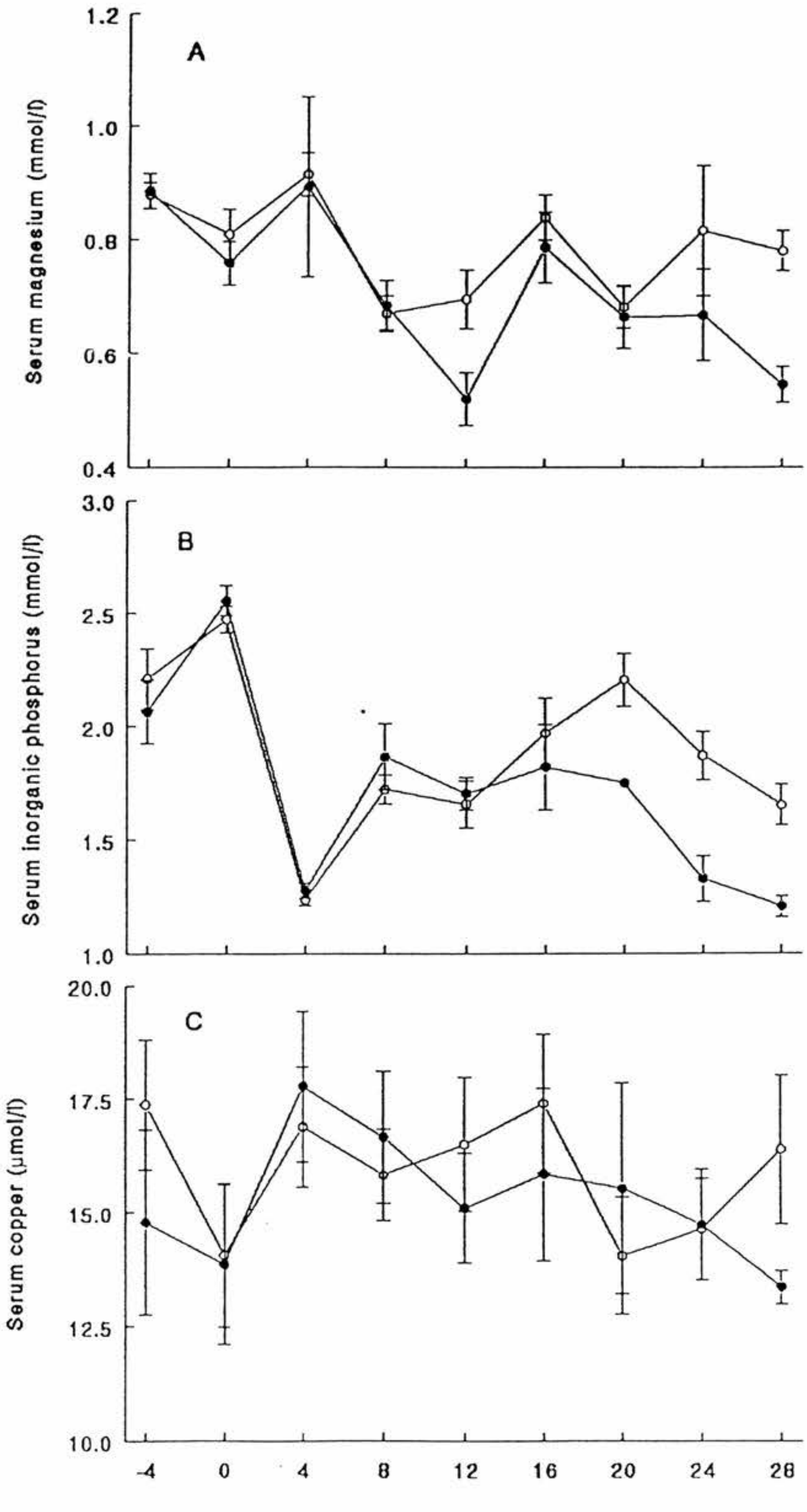


Figure 8.4.17. Mean values ± SE of serum magnesium (A), serum inorganic phosphorus (B) and serum copper (C) concentrations of the infected (●) and uninfected (O) groups of buffalo calves in experiment 4.

8.5 Experiment 5: Experimental infection of Nepalese hill buffaloes with low dose (400) of *F. gigantica* metacercariae

8.5.1 *Clinical data*

Except for calves 61 and 62 in which loss of condition and pallor of mucous membranes were observed from weeks 20 PI, all the buffalo calves appeared clinically normal and did not show any sign attributable to fasciolosis at any time during the experiment. All the infected and uninfected calves survived till the end of the experiment.

8.5.2 *Liveweight data*

There was no apparent difference between the groups up to 11 weeks after infection. Thereafter the infected buffaloes gained less weight than uninfected controls, and actually lost weight between 24 and 32 weeks after infection (Figure 8.5.1). Although, there appeared to be liveweight gain in infected buffaloes once more from 33 WPI, the mean liveweights still remained lower than those of the uninfected controls. The Mann-Whitney test showed a significant difference in the median liveweights of the infected and uninfected groups at 31, 32 and 34 WPI (Appendix Table 8.5.1).

Regression analysis of the liveweights against WPI (from 0 WPI to 35 WPI) revealed that the liveweight gains (i.e. slopes of the regression lines) of the infected buffaloes ranged from 0.572 to 1.447 kg/week with an average of 1.018 kg/week, while the uninfected buffaloes gained of 1.160 to 1.781 kg/week with an average of 1.666 kg/week (Appendix Table 8.5.2). Analysis of variance showed that there were significant differences between the slopes of the lines for the infected and uninfected groups (Figure 8.5.2 and Appendix Table 8.5.3).

There was a negative correlation between liveweight gain and terminal fluke burden (Figure 8.5.3)(r = -0.868, d.f. = 14, $P = < 0.0001$). This suggested that the liveweight gain of the infected calves was depressed at the rate of 6.5 g/week/fluke under the conditions of this experiment.

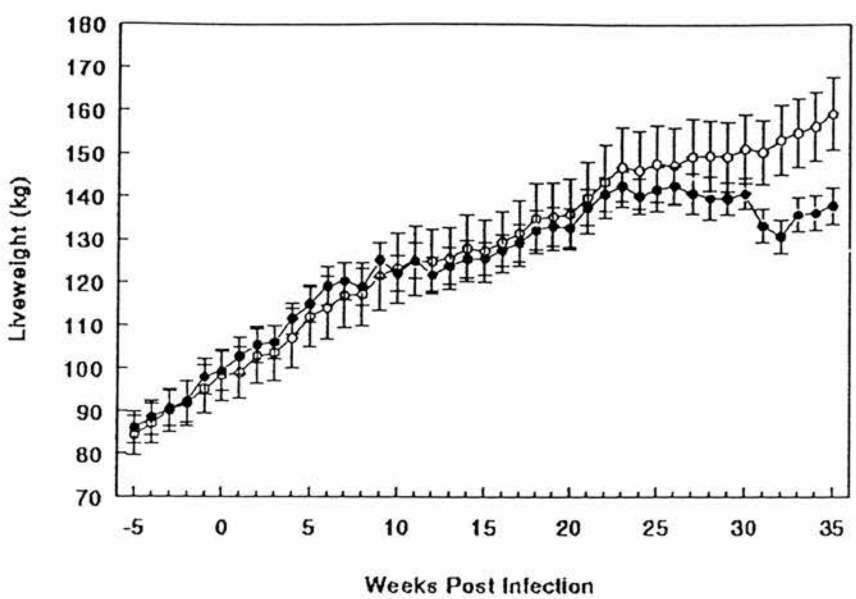


Figure 8.5.1. Mean liveweight ± SE of infected (●) and uninfected (O) buffalo calves in experiment 5.

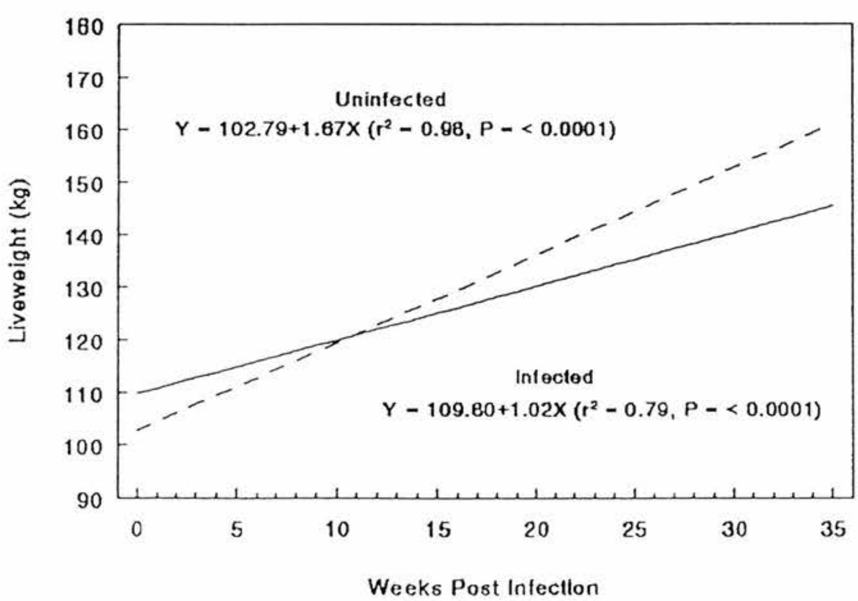


Figure 8.5.2. Regression lines of the liveweights of infected (—) and uninfected (--) groups of buffalo calves against weeks post infection. Slopes of these lines were significantly different ($F = 39.90$, d.f. = 14, $P = < 0.0001$).

8.5.3 *Carcass data*

After post mortem examination, carcasses of the infected and uninfected buffaloes were processed for the estimation of dressing percentage. The mean carcass weight and mean dressed carcass weight of the infected group were significantly less than those of the uninfected group (t = 2.27, d.f. = 14, P = 0.04 and t = 5.5, d.f. = 14, P = < 0.001 respectively). Also, the mean dressing percent of 37.50 for the infected buffaloes was significantly different compared to that of 42.76 for the uninfected controls (t = 4.84, d.f. = 14, P = < 0.001). The carcass data for the individual buffaloes are given in Appendix Table 8.5.5.

8.5.4 *Parasitological data*

The prepatent period ranged from 84 to 102 days with a mean of 95 days (Table 8.5.1). Although, there was a week to week variation in EPG and the highest mean count of 23 EPG occurred at 29 weeks after infection (Appendix Table 8.5.4), there appeared to be an exponential rise in EPG for 22 weeks after patency (Figure 8.5.4). Spearman's rank correlation coefficient showed that the faecal egg counts at the end of the experiment (35 WPI) were related to the number of flukes recovered post mortem (r_s = 0.881, d.f. = 6, P = 0.004).

Table 8.5.1. Some parasitological details of the buffalo calves in infected group in experiment 5.

Calf No.	Pre-patent period (days)	Fluke recovery		Mean (± SD) fluke size (mm)	
		No.	%	Length	Width
61	91	152	38.0	42.0 (± 2.6)	10.1 (± 0.5)
62	90	144	36.0	40.7 (± 2.3)	9.8 (± 0.6)
63	98	66	16.5	42.6 (± 3.5)	10.8 (± 0.7)
64	96	25	6.3	43.8 (± 2.6)	10.0 (± 0.7)
65	98	112	28.0	39.4 (± 2.8)	9.8 (± 0.6)
66	84	74	18.5	41.6 (± 4.2)	10.1 (± 0.7)
67	102	36	9.0	41.6 (± 2.6)	10.1 (± 0.5)
68	101	62	15.5	42.8 (± 2.5)	9.9 (± 0.6)
Mean	95	83.9	21.0	41.8	10.1

All the calves were slaughtered 35 weeks after infection.

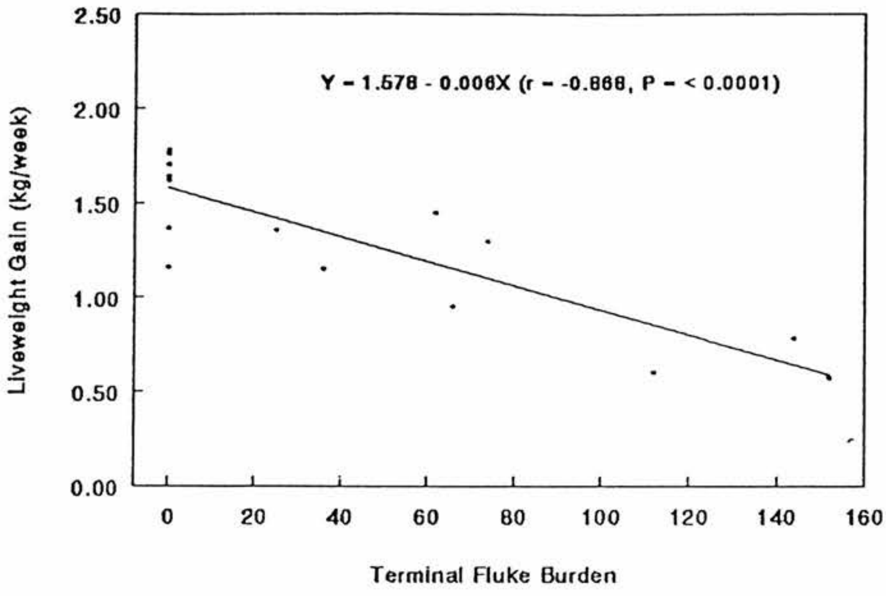


Figure 8.5.3. Regression line showing the relationship between the fluke burden and liveweight gain in 16 buffaloes in experiment 5.

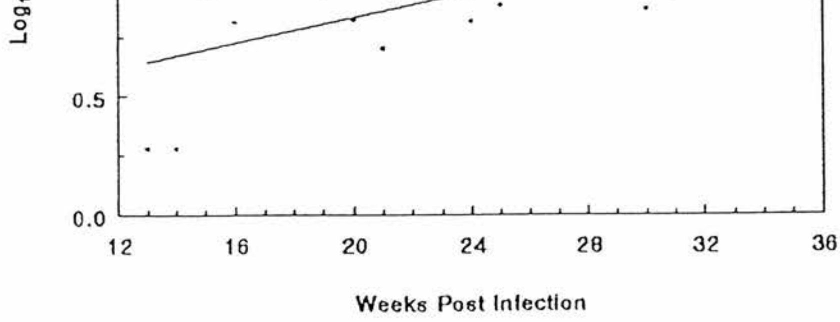


Figure 8.5.4. Regression line for the scatter plot of *F. gigantica* geometrical mean faecal egg counts ($\log_{10}$ EPG) of the infected buffalo calves in experiment 5.

All the flukes from the livers of the infected buffaloes at post mortem (35 WPI) were adults. Recovery was quite variable, ranging from 6.3 to 38 percent (Table 8.5.1). Spearman's rank correlation coefficient revealed that there was a negative correlation between the mean fluke length and fluke burden at the end of the experiment (r_s = -0.816, d.f. = 6, P = 0.014).

No flukes were recovered from any of the uninfected controls. Also, no other helminths were recovered from the rumen, abomasum, intestines or pancreatic ducts either of the infected or uninfected buffaloes.

8.5.5 *Pathological data*

The livers of the infected buffaloes were enlarged, with a mean weight of 1.919 kg, compared to the controls with a mean weight of 1.769 kg (Appendix Table 8.5.5). However, there was no significant difference in the mean weight of livers between the two groups (t = 0.91, d.f. = 14, P = 0.376). The gall bladders and hepatic bile ducts were consistently enlarged and the walls were thickened (Figure 8.5.5). Grossly, the fibrosis mainly affected the ventral lobe of the liver where the bile ducts were prominently distended on the visceral surface. The hepatic lymph nodes were also enlarged.

Histologically, there was mild to marked periportal and perilobular fibrosis within the parenchyma, and moderate to marked infiltration by mononuclear inflammatory cells particularly in periportal areas (Figure 8.5.6). Degeneration of hepatocytes and disruption of the hepatic parenchyma were also noted. Affected livers showed mild to moderate biliary hyperplasia particularly in the portal zones. One animal (calf 64) had a few large focal areas of parenchymal necrosis associated with marked haemorrhage and a surrounding zone of neutrophil polymorphs (Figure 8.5.6). In bile ducts and gall bladders containing flukes the epithelium was necrotic and eroded and other areas showed epithelial hyperplasia. There was marked mononuclear inflammatory cell infiltration within the lamina propria of gall bladders and the walls of gall bladders and bile ducts were fibrosed. There was no evidence of calcification of the bile ducts.

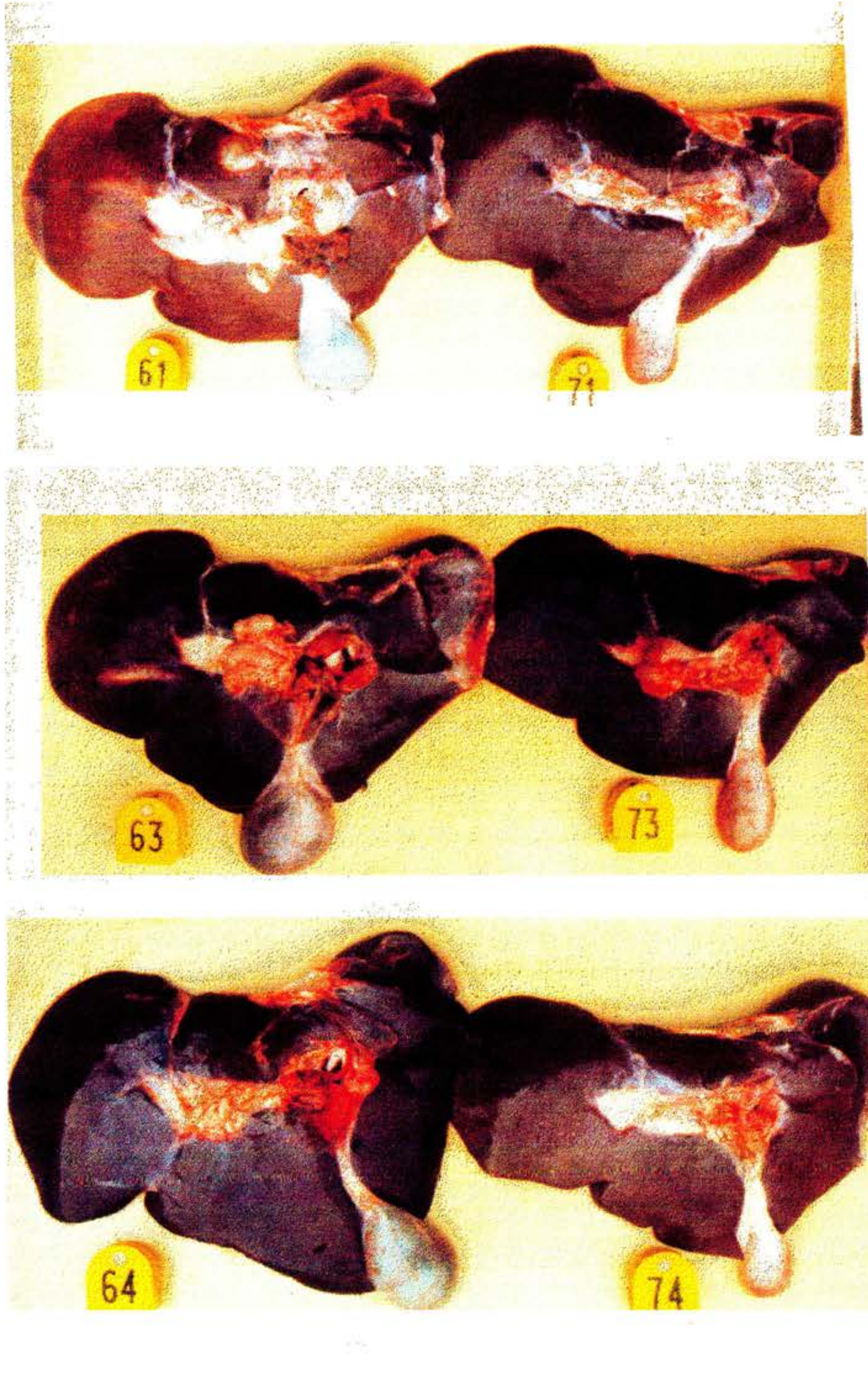


***Figure** 8.5.5.* Livers of infected (61, 63 and 64) and uninfected (71, 73 and 74) buffaloes. The buffaloes 61, 63 and 64 had burdens of 152, 66 and 25 *F. gigantica* respectively when slaughtered at 35 WPI. Note comparatively enlarged gall bladders and distended bile ducts in the infected livers.

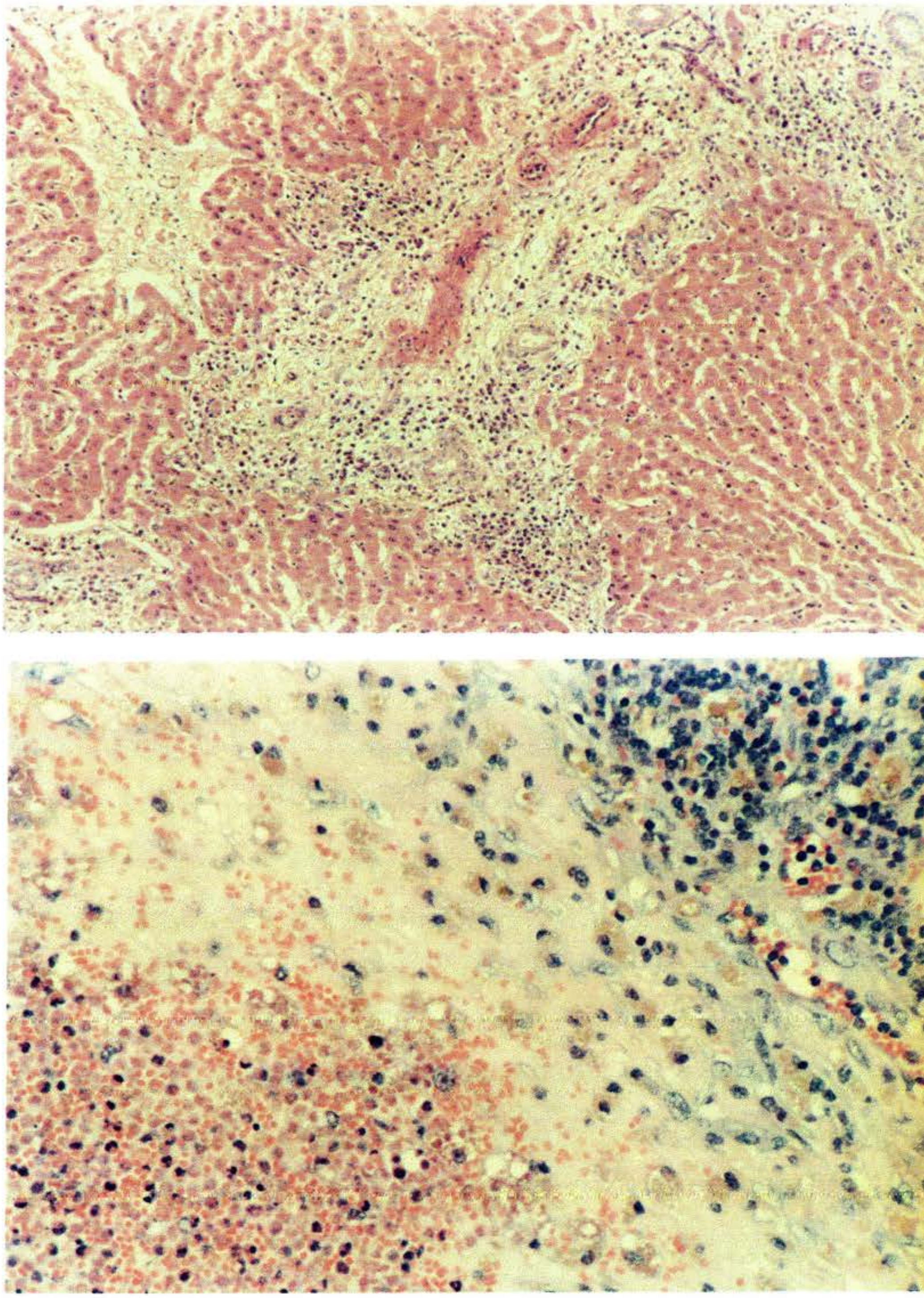


Figure 8.5.6. Photomicrographs of the liver sections of buffaloes 61 (top) and 64 (bottom). Top plate showing fibrosis and mononuclear inflammatory cells infiltration in periportal area (H&E stained, x130 magnification); Bottom plate showing area of parenchymal necrosis associated with haemorrhage, early fibrosis and infiltration of neutrophil polymorphs (H&E stained, x260 magnification).

8.5.6 *Haematological data*

In comparison to the control, the infected group exhibited a reduction in mean values for RBC counts, PCVs and haemoglobin concentrations which were more marked between 15 and 24 WPI (Figure 8.5.7). However, these values did not fall progressively in the infected buffaloes as the values began to rise from 23 WPI. The RBC counts regained to preinfection levels by 35 WPI, although they were still below those of the control group. The Mann-Whitney test showed that there were no significant differences between the median values of RBC counts, PCVs and haemoglobin concentrations of the two groups at any of the WPI determinations (Appendix Tables 8.5.6, 8.5.7 and 8.5.8 respectively). The regression of mean RBC counts, PCVs and haemoglobin concentrations (15 to 24 WPI) on terminal fluke burden, showed significant negative correlations ($r = -0.884$, d.f. = 6, $P = 0.004$; $r = -0.762$, d.f. = 6, $P = 0.028$; $r = -0.077$, d.f. = 6, $P = 0.025$ respectively).

There were no significant changes in MCV values as a result of the infection (Appendix Table 8.5.9), although comparatively higher mean values were noted in the infected group almost throughout the entire experimental period (Figure 8.5.8). There were erratic rises and falls in the mean MCH and MCHC values of the both groups, but these were more pronounced in the infected group (Figure 8.5.8). During the last few weeks (31 to 35 WPI), both these values were observed to be lower in the infected buffaloes, however, no significant differences were detected between the groups at any point during the course of the experiment (Appendix Tables 8.5.10 and 8.5.11).

There was a rise in total WBC counts from the 2 WPI in the infected buffaloes. Thereafter the mean WBC counts remained higher than those of the uninfected controls until the end of the experiment (Figure 8.5.9), although, the differences between the groups were not significant (Appendix Table 8.5.12). Similarly, the infected buffaloes had mild eosinophilia between 2 and 28 WPI, with the mean counts at their highest levels 5 weeks after infection (Figure 8.5.9). The differences in median values of the infected and uninfected groups were significant from 5 to 20 WPI (Appendix Table 8.5.13). There was no significant correlation between either mean total WBC (2-35 WPI) or mean eosinophil counts (5-20 WPI) with terminal fluke burden ($r = 0.411$, d.f. = 6, $P = 0.312$ and $r = 0.168$, d.f. = 6, $P = 1.000$ respectively).

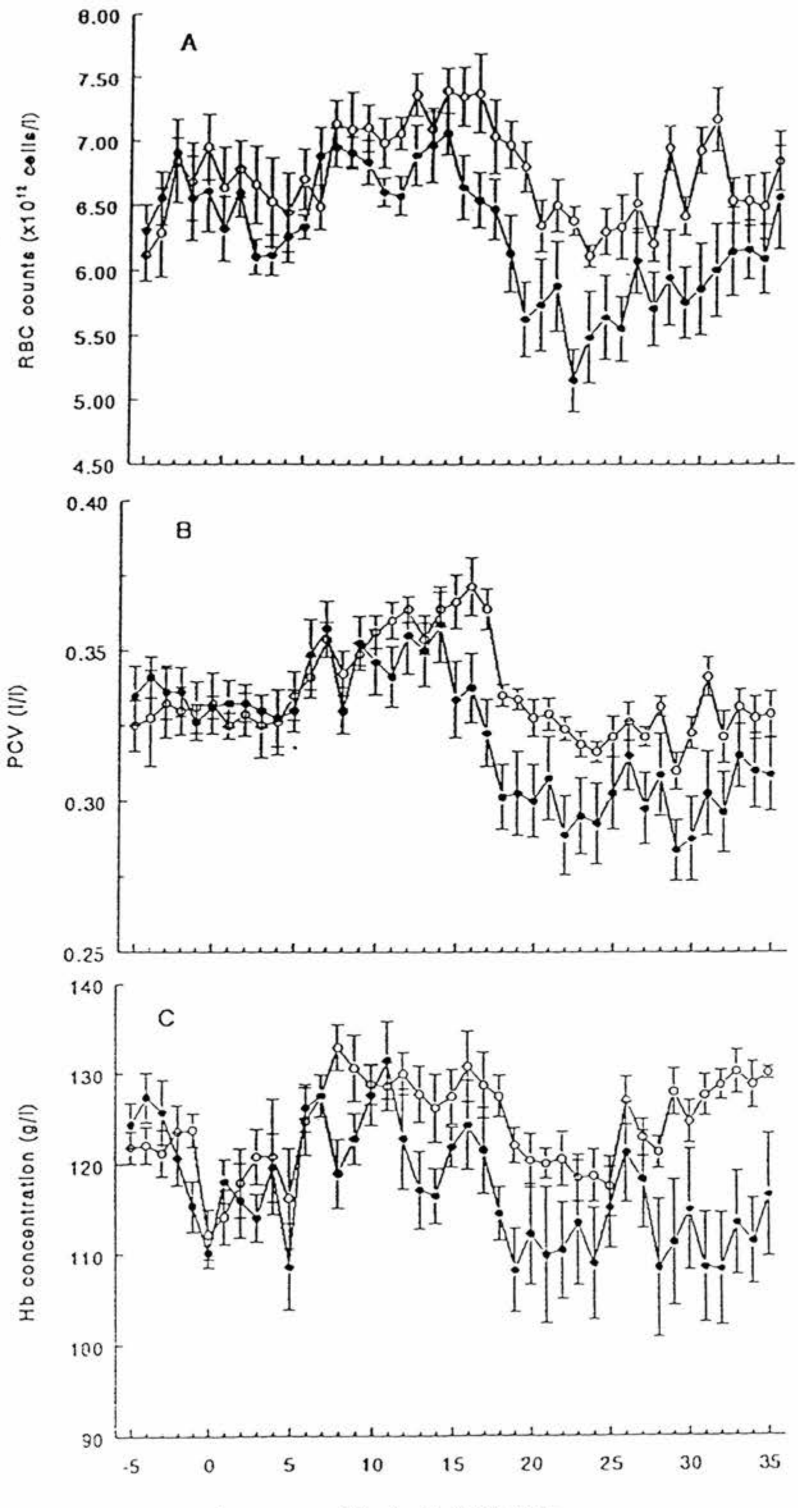


Figure 8.5.7. Mean values ± SE of RBC counts (A), PCV (B) and haemoglobin concentrations (C) of the infected (●) and uninfected (O) groups of buffalo calves in experiment 5.

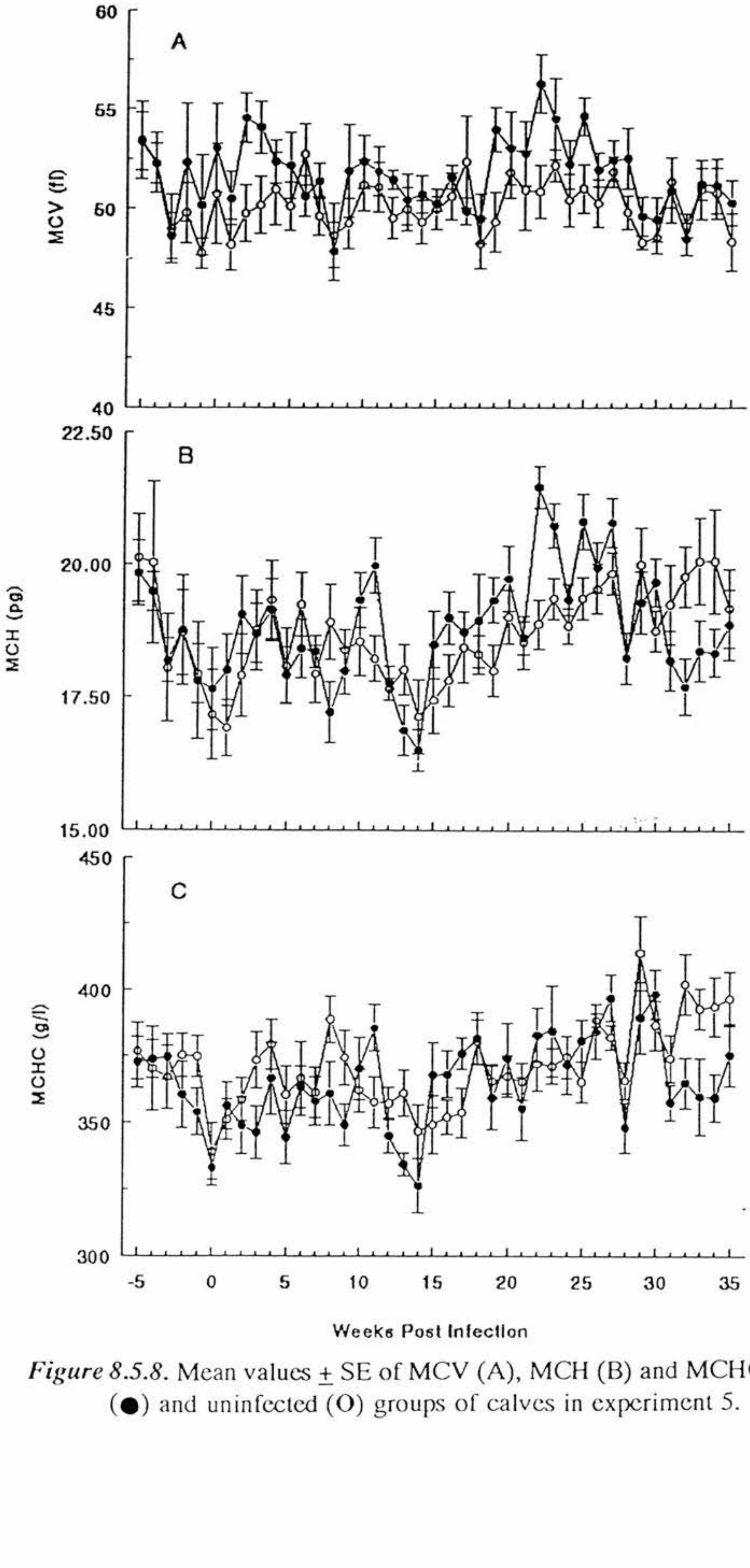


Figure 8.5.8. Mean values ± SE of MCV (A), MCH (B) and MCHC (C) of the infected (●) and uninfected (O) groups of calves in experiment 5.

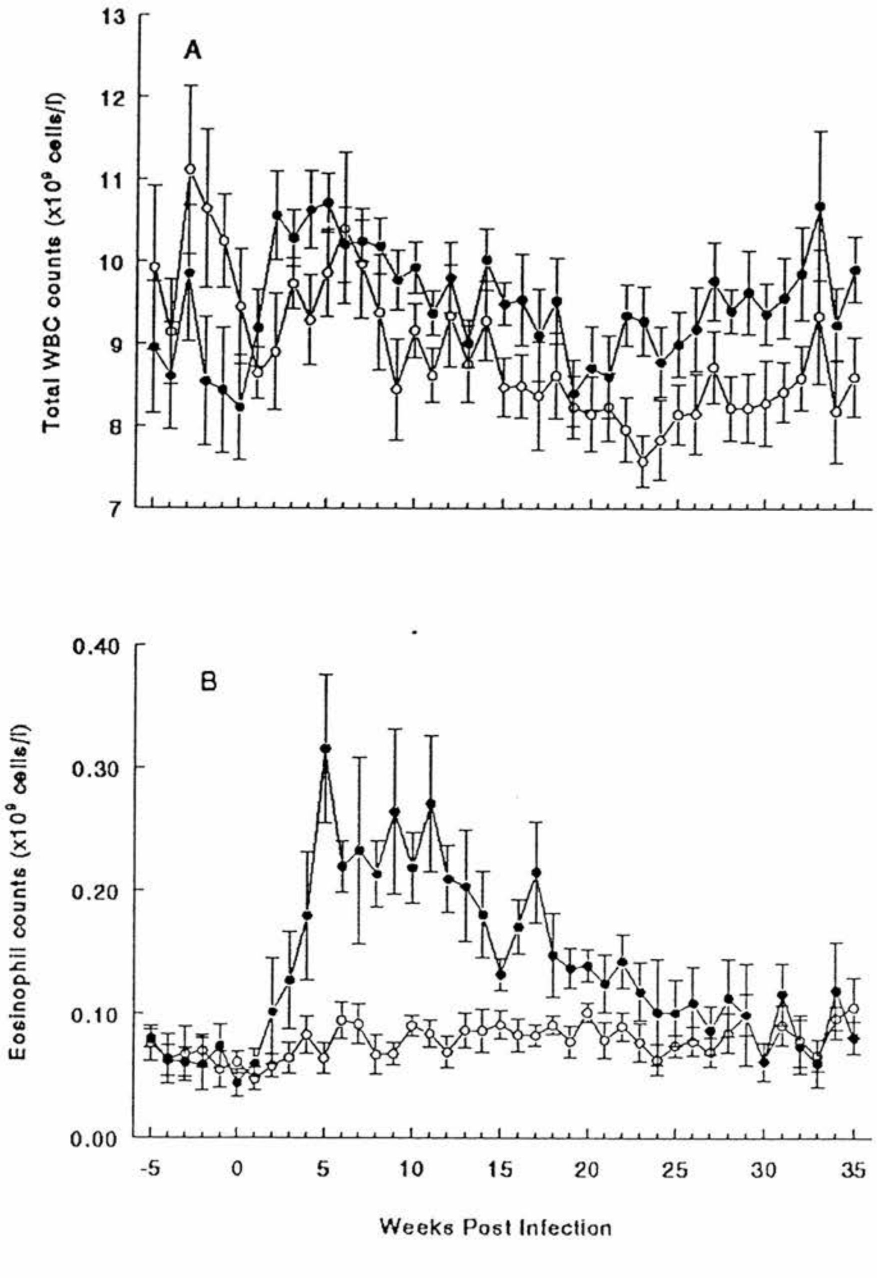


Figure 8.5.9. Mean values ± SE of total WBC (A) and eosinophil (B) counts of the infected (●) and uninfected (O) groups of calves in experiment 5.

8.5.7 *Biochemical data*

After an initial slight rise between 7 and 14 WPI, the mean total serum protein concentrations in the infected group declined progressively until 29 WPI (Figure 8.5.10). The levels tended to rise again reaching almost those of the uninfected controls from 30 WPI. Statistical comparisons showed that the median values of the two groups were significantly different between 20 and 29 WPI (Appendix Table 8.5.14). The regression of mean total serum protein concentration (from 20 to 29 WPI) against terminal fluke burden revealed a marginally significant negative correlation ($r = -0.690$, d.f. = 6, $P = 0.059$).

There was a general decline in serum albumin concentrations in the infected buffaloes from 11 until 27 WPI; thereafter the levels tended to rise (Figure 8.5.10). A similar pattern was also observed in the uninfected buffaloes, however, the median values of the infected buffaloes were significantly lower than those of the uninfected controls from 21 WPI till the termination of the experiment (Appendix Table 8.5.15). There was a significant negative correlation between mean serum albumin concentration (21-35 WPI) and terminal fluke burden ($r = -0.819$, d.f. = 6, $P = 0.013$).

The infected buffaloes had a transient, but significant rise in the mean serum globulin concentrations between 7 and 14 WPI (Figure 8.5.11 and Appendix Table 8.5.16). The mean concentrations declined below those of the uninfected controls from 20 WPI, but the differences between the groups were not significant (Appendix Table 8.5.16).

The ratios of serum albumin to serum globulin were generally observed to be lower in the infected buffaloes compared to those in the uninfected controls throughout the experimental period (Figure 8.5.11). The median values of the two groups, however, were significantly different only between 9 and 15 WPI (Appendix Table 8.5.17).

While the uninfected controls maintained a fairly uniform level of serum GLDH throughout the experiment, the levels rose in the infected buffaloes from 3 WPI with a peak at 11 WPI (Figure 8.5.12). The Mann-Whitney test indicated that the differences were significant from 4 WPI till the termination of the experiment (Appendix Table 8.5.18). There was not a significant correlation between the mean GLDH values (4-15 WPI) and terminal fluke burdens ($r = 0.450$, d.f. = 6, $P = 0.263$).

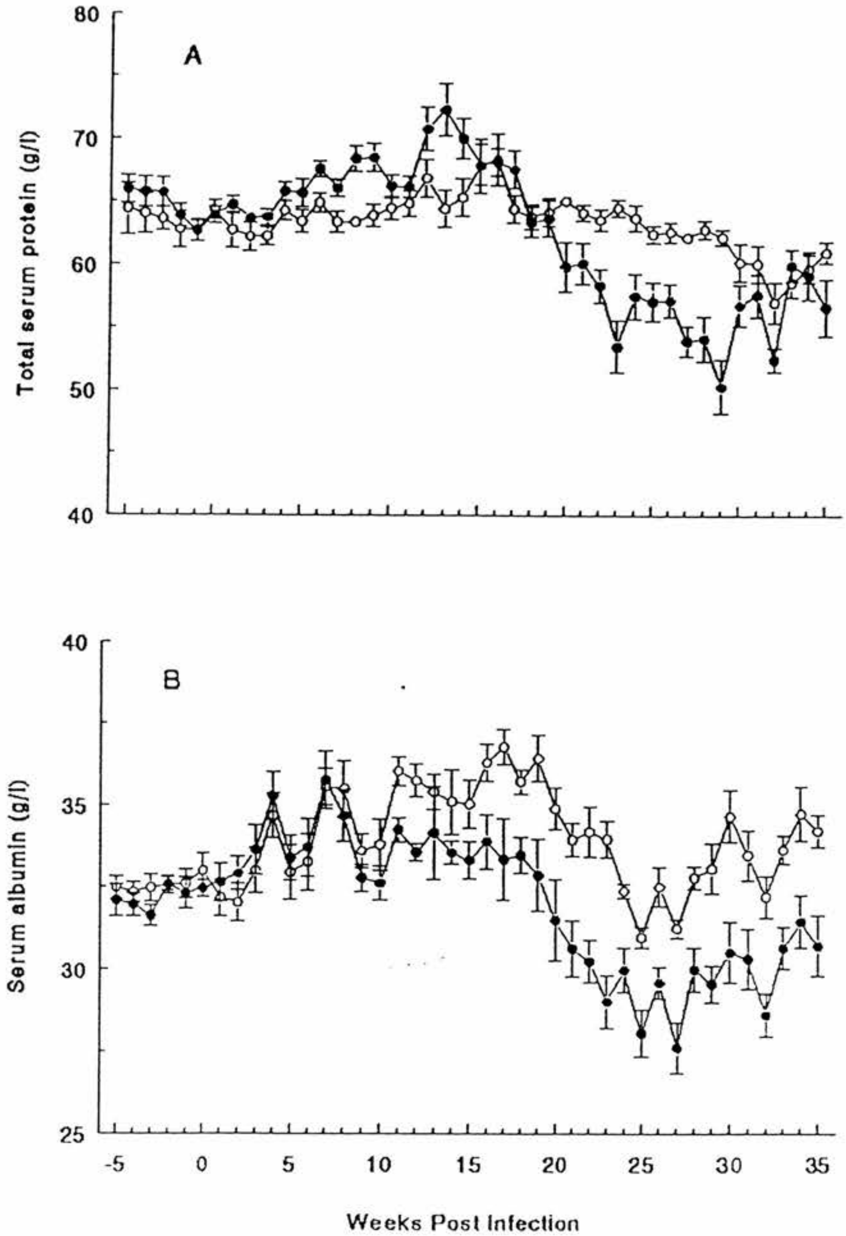


Figure 8.5.10. Mean values ± SE of serum total protein (A) and serum albumin (B) concentrations of the infected (●) and uninfected (O) groups of calves in experiment 5.

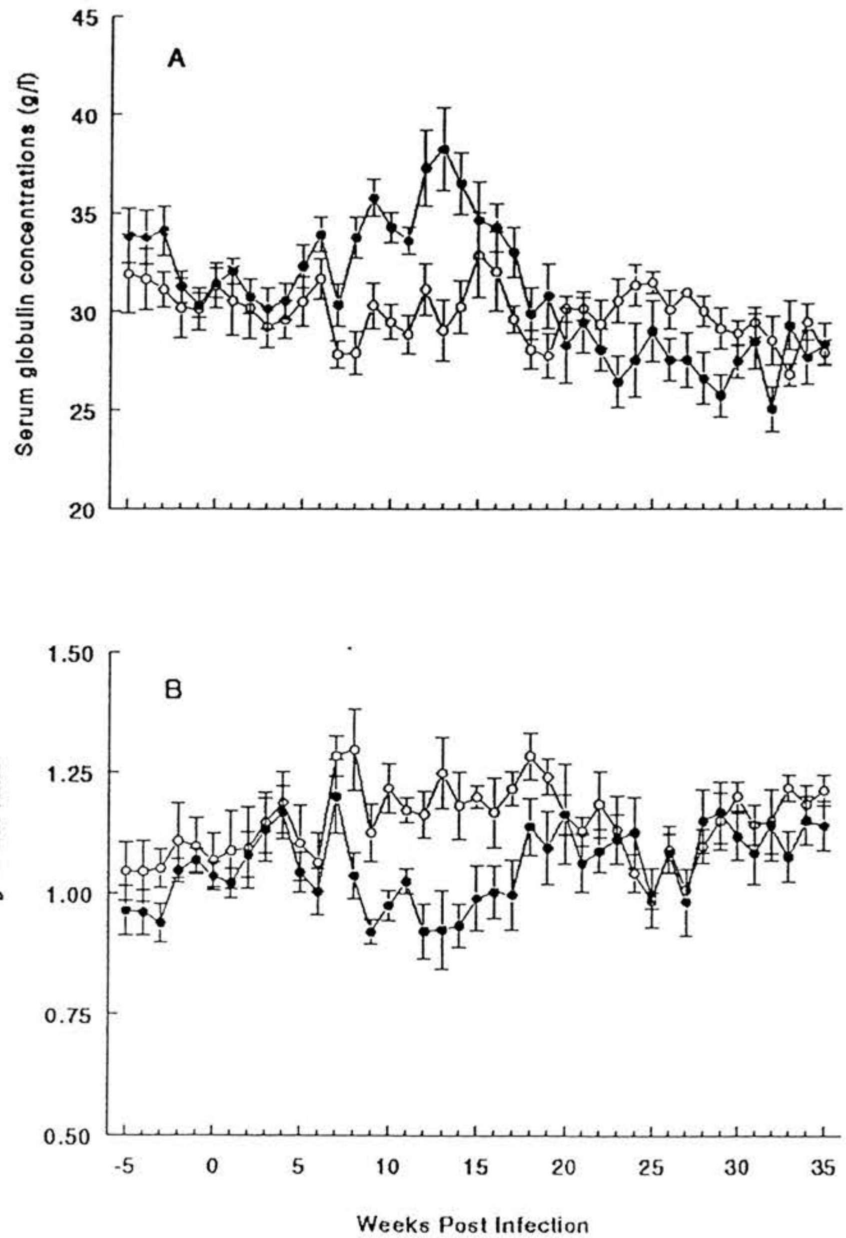


Figure 8.5.11. Mean values ± SE of serum globulin concentrations (A) and serum albumin to serum globulin ratios (B) of the infected (●) and uninfected (O) groups of calves in experiment 5.

GGT levels in the infected buffaloes first rose at 6 WPI. Thereafter several sharp rises and falls with the highest peak at 18 WPI were observed (Figure 8.5.12). Statistical comparisons showed that the GGT levels in the two groups of buffaloes were significantly different from 11 WPI until the end of experiment (Appendix Table 8.5.19). There was no significant correlation between the mean GGT levels (11 to 28 WPI) and terminal fluke burden ($r = 0.253$, d.f. = 6, $P = 1.000$).

By comparison with the controls the infected group had generally lower levels of serum calcium, magnesium, inorganic phosphorus and copper from 8, 12, 12 and 4 WPI respectively (Figures 8.5.13 and 8.5.14). Serum potassium levels were declined between 12 and 24 WPI but thereafter rose above the levels of the uninfected controls (Figure 8.5.13). There was an erratic trend in the levels of serum sodium concentrations (Figure 8.5.13). Significant differences between the mean values of the two groups were detected for calcium at 24 WPI, sodium at 35 WPI, magnesium at 12 and 16 WPI, inorganic phosphorus at 16 WPI and copper from 24 WPI till the end of the experiment (Appendix Table 8.5.20).

8.5.8 *Agar gel diffusion assay*

Control buffalo calves all gave negative results but all infected buffaloes became positive between 5 and 7 WPI, and remained strongly positive until 16 WPI (Appendix Table 8.5.21). Thereafter, sera from the infected calves were sometimes negative intermittently, and 30 WPI, all the infected calves gave negative results, except for calf 65 which remained positive until 34 WPI.

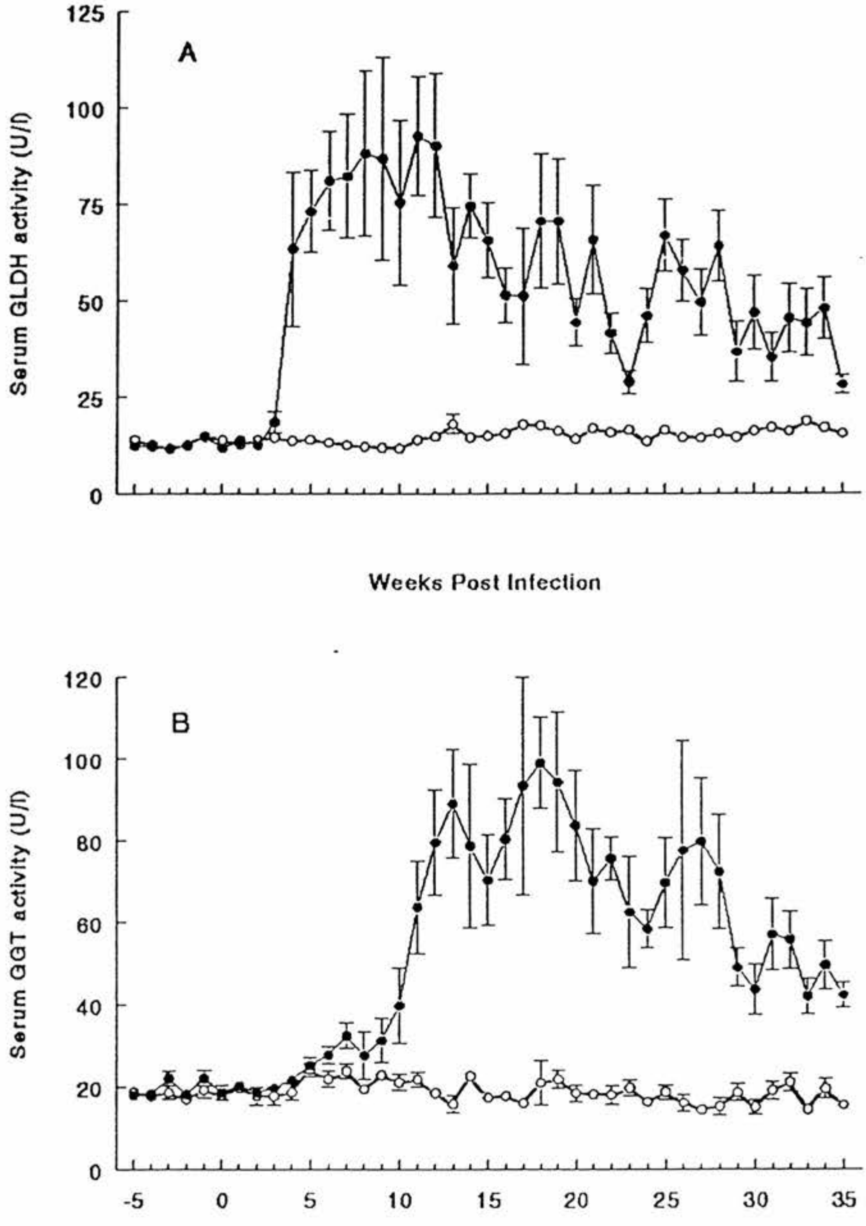


Figure 8.5.12. Mean values ± SE of the activities of GLDH (A) and GGT (B) in the sera of infected (●) and uninfected (O) groups of calves in experiment 5.

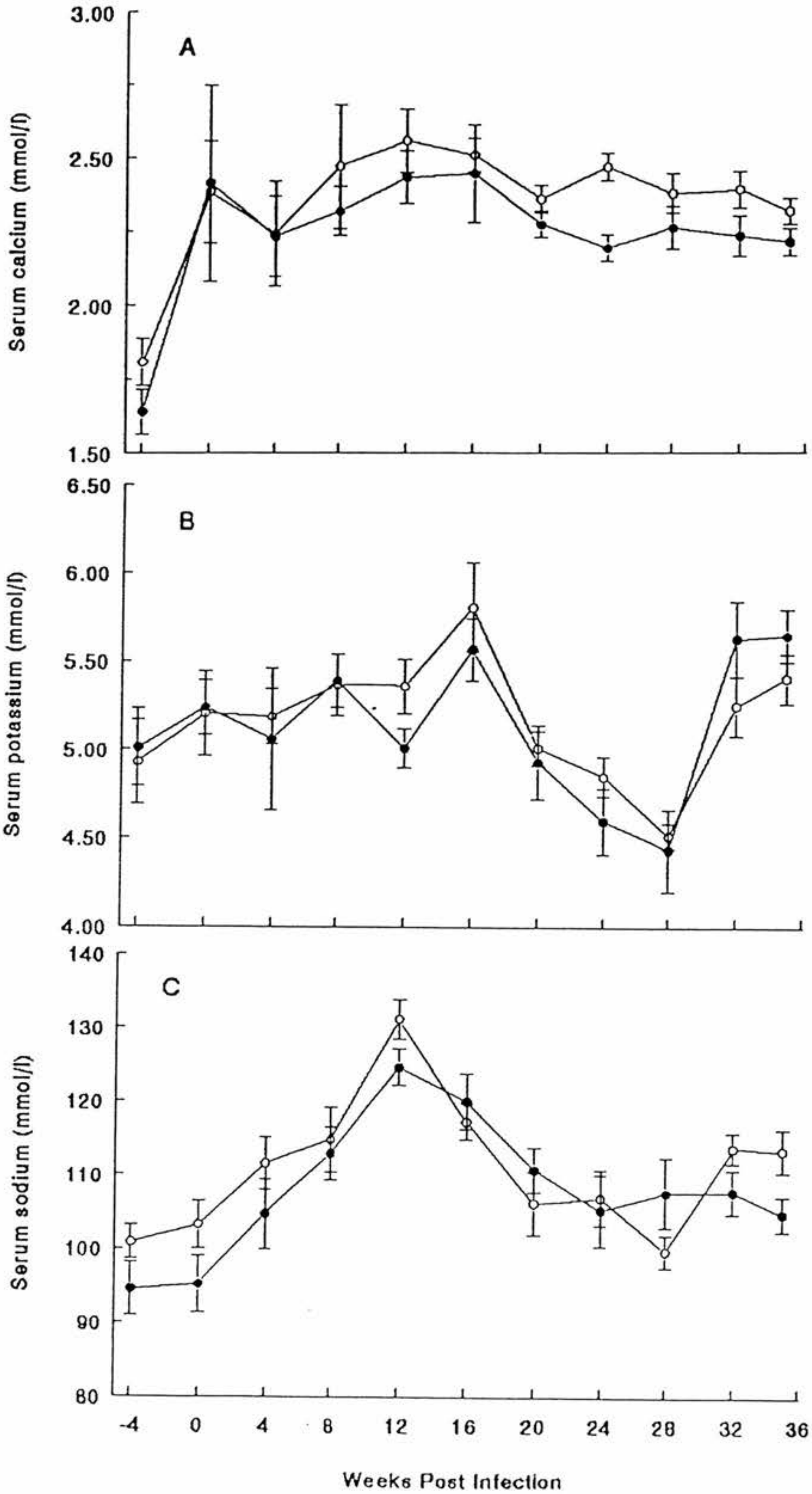


Figure 8.5.13. Mean values ± SE of serum calcium (A), serum potassium (B) and serum sodium (C) concentrations of the infected (●) and uninfected (O)groups of calves in experiment 5.

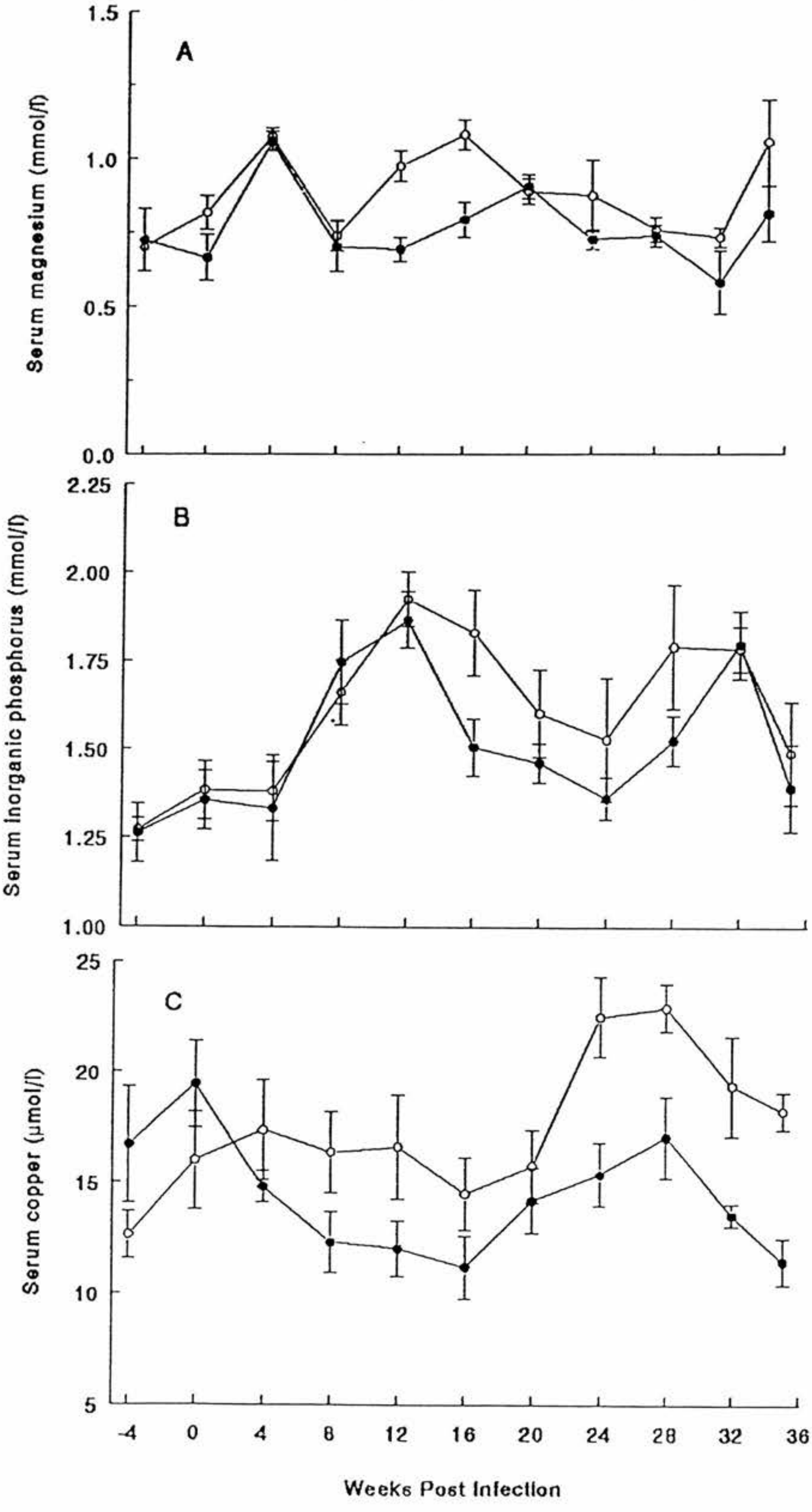


Figure 8.5.14. Mean values ± SE of serum magnesium (A), serum inorganic phosphorus (B) and serum copper (C) concentrations of the infected (●) and uninfected (**O**) groups of calves in experiment 5.

CHAPTER NINE

DISCUSSION

9.1 Clinical Observations

9.1.1 *Comparative pathogenicity of F. gigantica and F. hepatica in sheep (Experiment 1)*

This pilot study which was carried out at CTVM, was designed with two main objectives in view. The first of these was to establish the techniques required for the pathogenesis studies carried out at PAC in Nepal, while the second was to compare the pathogenic effects of *F. gigantica* and *F. hepatica* infections in sheep.

The conclusions that may be drawn from this experiment are severely limited by the individual variation and the small numbers of experimental animals involved. There was a clear evidence, however, that *F. gigantica* is more pathogenic than *F. hepatica* in sheep. Two sheep, harbouring 80 (1.7 flukes/kg of initial liveweight) and 181 (7.0 flukes/kg) *F. gigantica* died due to subacute fasciolosis at 14 and 15 WPI respectively, while clinical chronic fasciolosis developed in one sheep which had burden of 83 (1.7 flukes/kg) *F. gigantica.* On the other hand, *F. hepatica* infected sheep, even those with burdens of 203 (5.7 flukes/kg) and 247 (8.5 flukes/kg) flukes survived until the end of the experiment, and only one sheep which had a burden of 247 (8.5 flukes/kg) flukes showed transient clinical signs characterised by loss of appetite around 10 WPI.

These findings supported the views of Sogoyan (1956) and Ogunrinade (1984) that the number of parasites required to present the same type of fasciolosis syndrome in the same host species is lower for *F. gigantica* than *F. hepatica.* Sogoyan (1956) compared the pathogenicity of *F. gigantica* and *F. hepatica* in sheep and found that 10 sheep harbouring 33 to 265 adult *F. gigantica* died between 77 and 88 days after infection, whereas infection of 122 to 870 adult *F. hepatica* did not result in mortality in sheep for up to 156 days. In the study conducted by Ogunrinade (1985), 100 to

300 *F. gigantica* caused fatalities from the acute fasciolosis syndrome 11 to 12 weeks after infection, while Pullan et al. (1970) could only produce acute fasciolosis in sheep with burdens in excess of 1,400 *F. hepatica*. Likewise, Ross et al. (1967) found that 1,000 to 2,500 *F. hepatica* were needed to produce acute fasciolosis in sheep. Ogunrinade (1984) suggested that the longer period of migration of *F. gigantica* in the liver together with its larger size probably accounts for the greater pathogenicity of this species than of *F. hepatica* in small ruminants.

9.1.2 *Experimental F. gigantica infection in Nepalese Baruwal sheep (Experiment 2)*

In this pilot experiment, the infective doses (number of metacercariae/kg of initial liveweight) were the same as those in the goat experiment discussed below. The aim of these two experiment was to produce fasciolosis in sheep and goats with approximately similar fluke burdens in relation to the liveweights so that a comparison could be made between the pathogenesis in these two species. Furthermore, as the infective doses of the Nepalese strain of *F. gigantica* in these experiments were similar to those used in the study on comparative pathogenicity of *F. gigantica* (Kenyan strain) and *F. hepatica* (UK strain) in sheep, it was thought that the pathogenicity of the two different strains of *F. gigantica* could be compared indirectly. Unfortunately, these comparisons could not be made because of large differences in fluke recoveries between the two experiments.

The absence of clinical signs of fasciolosis in the sheep infected with a mean burden of 7.3 *F. gigantica* (0.3 fluke/kg) agreed with the findings of Hammond (1973). He also did not observe any clinical signs, although his sheep had a heavier mean burden of 37.3 flukes (0.6 fluke/kg).

9.1.3 *Experimental F. gigantica infection in Nepalese hill goats (Experiment 3)*

The burdens of 23 (1.3 flukes/kg) to 68 (3.3 flukes/kg) flukes produced clinical chronic fasciolosis in goats characterised mainly by a rough coat, pallor of the mucous membranes and general loss of condition. The clinical signs were not evident in goats harbouring 4 (0.2 flukes/kg) and 7 (0.4 flukes/kg) flukes. The severity of the pathogenic effects appear to be dependent on the fluke burden in relation to body weight rather than the total number of flukes recovered. For example, the presence of 55 flukes (3.6 flukes/kg) caused death about 33 weeks after infection in one goat while another goat which had a burden of 68 flukes (3.3 flukes/kg) survived until the end of the experiment (35 WPI).

Hammond (1965) reported that goats with fluke burdens of 42-73 *F. gigantica* died between 94 and 110 days after infection, while in sheep with up to 47 flukes, Wiedosari and Copeman (1990) observed no clinical signs. As the liveweights of the animals used in these experiment were not indicated, no comparisons of the results are possible. However, these findings agreed with those of Ogunrinade (1984) who showed that *F. gigantica* infection in goats was more severe than in sheep resulting in shorter survival time of goats after infection compared to sheep given the same infective doses. It appears, therefore, that fasciolosis may be a more severe disease in goats than in sheep.

9.1.4 *Experimental F. gigantica infection in Nepalese hill buffaloes (Experiments 4 and 5)*

Under the experimental conditions, no clinical signs were observed in the buffalo calves which harboured 112 (1 fluke/kg) or fewer flukes, while burdens in excess of 144 *F. gigantica* ($\geq$ 1.3 flukes/kg) resulted in clinical fasciolosis.

A chronic disease, characterised by progressive weakness and pallor of mucous membranes featured those calves which had burdens between 144 and 587 flukes (1.3 to 4.9 flukes/kg). Two of these calves became moribund around 26 WPI but the remaining 4 survived until the end of the experiments, although they were in poor condition and anaemic. Literature on experimental fasciolosis in buffaloes could not be traced for comparison. Except for depraved appetite and submandibular oedema, however, other clinical signs observed in the present experiments were similar to those stated in a review by Sukhapesna (1992) for chronic fasciolosis in buffaloes. Depraved appetite which is usually associated with a deficiency of protein and some minerals (Blood and Radostitis, 1989), has been recognised by Nepalese farmers as a prominent feature of chronic fasciolosis in ruminants; thus the disease is commonly known as *MATE*, which means soil (earth) eater.

The presence of 721 to 847 flukes (7.4 to 9.1 flukes/kg) caused death of calves between 14 and 18 WPI. The syndrome produced was similar to that described by Ogunrinade (1983) as subacute fasciolosis in cattle. However, neither submandibular oedema nor a severe terminal diarrhoea which were observed in one buffalo calf in the present study, were seen in his cattle. Sewell (1966) used 20,000 metacercariae giving rise to about 7,000 young flukes, to cause a 2 year old (191 kg initial liveweight) zebu steer to die of subacute fasciolosis 11 WPI, while Ogunrinade (1983) showed that only 5,000 metacercariae resulting in worm burdens in excess of 1,000 were enough to cause death of his 3-6 year old (250-350 kg initial liveweight) cattle between 19 and 22 WPI. Nevertheless, as the experimental cattle used by these workers were older and heavier than the buffalo calves used in the present study, any direct comparison of the clinical findings is not justifiable.

In the present study, one calf with a burden of 587 *F. gigantica* (5.6 flukes/kg) died 13½ WPI due to bacillary haemoglobinuria which is caused by the toxin of *Clotridum novyi* type D. Jaundice has been reported to occur in the later stages of subacute (Sewell, 1966) and chronic (Basu and Bandyopadhyay, 1986) fasciolosis in cattle. However, the sudden onset of this together with shallow and laboured respiration observed in the buffalo calf, are characteristics of bacillary haemoglobinuria resulting from the severe haemolysis which is caused by a haemolytic toxin formed by the organism (Janzen, Orr and Osborne, 1981). Although, haemoglobinuria was not observed before death of the buffalo calf, red urine was found in the urinary bladder at necropsy.

There are several reports on the association of *Fasciola* spp. as precipitating factor of black disease which is caused by the toxin of *Cl. novyi* type B (Bagadi, 1970; Bagadi and Sewell, 1973). Although, a similar association of *Fasciola* spp. with bacillary haemoglobinuria has been suggested (Janzen et al., 1981), the evidence is as yet incomplete yet (Blood and Radostitis, 1989). Thus the findings of the present study appear to be the first report of bacillary haemoglobinuria in buffaloes and indicates that infection with *Fasciola* spp. may indeed precipitate bacillary haemoglobinuria in animals.

9.2 Observations on Liveweight

9.2.1 *Comparative pathogenicity of F. gigantica and F. hepatica in sheep (Experiment 1)*

At 12 WPI, there was no gain in the mean liveweight of *F. gigantica* infected sheep which had mean burdens of 94.5 flukes (2.3 flukes/kg) while *F.hepatica* infected sheep with mean burdens of 155.5 flukes (3.8 fluke/kg) gained 1.125 kg during the same period. At the end of the experiment (20½ WPI), two *F. gigantica* infected surviving sheep which had a mean burden of 58.5 flukes (1.3 fluke/kg) gained only 1.500 kg of mean liveweight in comparison to 3.750 and 7.275 kg gained by *F. hepatica* infected and uninfected controls respectively. Thus, the results of the present study indicate that liveweight gain in infected sheep is more severely depressed by *F. gigantica* than *F. hepatica*. It should be emphasised, however, that the numbers of the sheep infected with each *Fasciola* spp. were not large enough to draw a firm conclusion on this point.

9.2.2 *Experimental F. gigantica infection in Nepalese Baruwal sheep (Experiment 2)*

Under the experimental conditions and infective doses given, the infected sheep gained less weight than the uninfected controls over the period from 0 to 35 WPI (Appendix Table 8.2.2). This finding is rather surprising, since no flukes were recovered from 2 of the 6 infected sheep and 4 sheep had a mean burden of only 7.3 flukes (0.3 flukes/kg).

Hammond (1973) observed a significantly lower liveweight gain in the infected sheep with a mean burden of 37.3 *F. gigantica* (0.6 flukes/kg). The differences in liveweight gain between his infected and uninfected groups of sheep arose, almost entirely, during the period 10-15 WPI. In the present study, however, depression of the liveweight gain was more pronounced after 21 WPI which followed a change in diet of these sheep; the supplement of *Ficus* spp. leaves was changed to *Prunus serasoides* and bamboo leaves from 19 WPI. Although the crude protein contents of these tree fodders vary between 11-15% of the dry matter, *P. serasoides* and bamboo leaves are considered to be poor quality fodder because of a lower dry matter intake by animals (Mahato, Subba and Tamang, 1989). It seems possible, therefore, that in the present study, the change in the diet from better to poor quality fodder affected the capacity of the infected sheep to withstand the pathogenic effects of the infection, resulting in a noticeable depression in their liveweight gain.

9.2.3 *Experimental F. gigantica infection in Nepalese hill goats (Experiment 3)*

The results suggested that *F. gigantica* may cause a significant reduction in weight gain in goats even at the low level of fluke burden and gave no support to a somewhat surprising belief of Nepalese farmers that low levels of fluke infection actually improve the condition of their animals. The commencement of the weight loss appeared to coincided with the entry of the flukes in the bile ducts at 11-12 WPI. The absolute reduction in liveweight was found to significantly correlate with the fluke burdens, each fluke reducing liveweight gain by about 6 g per week.

9.2.4 *Experimental F. gigantica infection in Nepalese hill buffaloes (Experiments 4 and 5)*

In the both experiments, the liveweight gain of the infected buffaloes was distinctly affected. The reduced rate of liveweight gain commenced much earlier in calves in experiment 4 which had heavier fluke burdens than in those in experiment 5. In experiment 4, however, each fluke reduced the weekly liveweight gain by only about 2.6 g per fluke, while in the experiment 5, this figure was 6.5 g per fluke. It seems, therefore, that a longer infection period with smaller fluke burden has a proportionally greater effect on the depression of liveweight gain than a shorter infection period with larger fluke burden.

9.3 Observations on Carcass Yield

These observations, which mainly included weighing the whole and dressed carcasses, were made only in experiments 2, 3 and 5 in order to evaluate the effect of chronic fasciolosis on carcass yield in sheep, goats and buffaloes. The present study showed that the mean dressing percentage of infected sheep, goats and buffaloes were reduced by 8.3%, 9.6% and 12.4% respectively than those of their respective controls, although the differences were only significant in goats and buffaloes. Similarly, the infected carcasses had less abdominal fat. These observations suggested that measurement of body weight alone may underestimate the extent of production losses in ruminants.

9.4 Parasitological Observations

9.4.1 *Prepatent period*

The prepatent periods for *F. gigantica* infections as indicated by the appearance of ova in faeces of the infected sheep, goats and buffaloes were found to be 96.7 ± 6.7, 90.2 ± 4.7 and 96.1 ± 5.1 days respectively. Analysis of variance showed that these figures which closely agree with previous estimates in sheep (Hammond, 1973; Ajanusi, Ogunsusi, Njoku and Gyang, 1988) and cattle (Sewell, 1962; Hammond, 1970), were not significantly different from each other ($F = 1.876$, d.f. = 2, $P = 0.1734$). Hammond (1970) observed a mean prepatent period of 95.1 and 91 days for *F. gigantica* infections in sheep and calves respectively, while Sewell (1962) gave a mean of 93 days for his calves. Longer or shorter prepatent periods have been recorded in sheep and cattle by other authors (Davtyan, 1953; Guralp, Ozcan and Simms, 1964; Dixon, 1965; Ogunrinade, 1983). These variations may simply be due to the different techniques employed.

9.4.2 *Faecal Fasciola egg counts*

The counts of *F. gigantica* eggs in the faeces were never very high in any animal, except a few buffalo calves in experiment 4. It was noted that on two occasions (23 and 28 WPI), *F. gigantica* eggs were seen in the faeces of sheep 13, from which no flukes were recovered at necropsy. The egg counts in sheep reported by Hammond (1970) were much higher than were seen in sheep and goats in the present work. Although his sheep had higher fluke burdens, this difference may also have been due to differences in egg counting technique, as Morel (1987) showed that the sedimentation technique used by Hammond gave consistently higher EPG values than differential flotation.

The faecal egg counts in buffalo calves in experiments 4 and 5 rose exponentially until the experiment was terminated 27 and 35 WPI, however, those in the sheep and goats in experiment 2 and 3 fell to low levels after 20 WPI. Although, Bitakaramire (1969) reported an exponential rise in the faecal egg counts in cattle over the first 15 weeks of patent infections, this was not observed by Sewell (1966), Hammond (1970) and Ogunrinade (1983). The decline in faecal *F. gigantica* egg counts in cattle from 25-30 WPI is well recognised and considered to be due to nutritional deficiencies in the flukes because of the increasing fibrosis and calcification of bile ducts (Sewell, 1966; Hammond and Sewell, 1975). Although, this calcification has not been reported in sheep or goats, Hammond (1973) noted that his sheep had a few dead and degenerating flukes in the liver when the experiment was terminated 29 WPI. It is possible, therefore, that the decline in the faecal egg counts observed in sheep and goats in the present work resulted from death of some flukes before 20 WPI.

9.4.3 *Fluke recovery*

In the present work, great variations in fluke recovery within the same and between experiments were observed. Except for experiments 1 and 4, the recoveries were generally low, varying between 0.0-8.0% in sheep (experiment 2), 2.2-36.8% in goats (experiment 3) and 6.3-38.0% in buffaloes (experiment 5); 2 of 6 infected sheep in experiment 2 had no flukes at necropsy. By comparison, Hammond (1973) reported a mean recovery of 62% in Merino X Corriedale sheep and Hammond and Sewell (1974; 1975) obtained a mean recovery of 61.3% in cattle between 80-140 days after infection. However, there are reports of lower recoveries, such as 10-22% in West African Dwarf sheep (Ogunrinade,1984), 0-22% in Javanese thin tailed sheep (Wiedosari and Copeman, 1990) and 12-30% in West African Dwarf goats (Ogunrinade. 1984). From their own observations taken together with those of the earlier authors, Hammond and Sewell (1975) concluded that the burdens observed at post mortem examination will vary due to several independent factors including the dose and infectivity of the metacercariae and the duration of infection. They further suggested that the strain of the parasite, and the breed, age, nutrition and management of the host may be involved.

Wiedosari and Copeman (1990) concluded that the lower fluke recovery in their Javanese thin tailed sheep was due to a high innate resistance of these sheep to *F. gigantica*. It seems unlikely, however, that the Nepalese Baruwal sheep, Nepalese hill goats and Nepalese hill buffaloes which were used in the experiments 2, 3 and 5 respectively, were less susceptible to *F. gigantica* infection, since a higher mean recovery of 40% (25.5-56.5%) was achieved in buffaloes in experiment 4. The higher fluke recovery from the buffaloes in experiment 4 than those in experiment 5 suggested that the metacercariae used in the later experiment were possibly less infective. It has been shown by Davtyan (1956), with *F. gigantica* and by Boray (1963), with *F. hepatica* that the infectivity of metacercariae is decreased by a higher temperature during their development as larvae. The metacercariae used in the experiments 2, 3 and 5 were produced in laboratory infected snails which were maintained in aquaria at 25 ± 2° C, while those used in the experiment 4 were obtained from snails with mature infection which were collected from a natural habitat where the maximum and minimum air temperature varied between 10-22° C and 0.5-15° C respectively during possible period of their development (November-December). The constant higher temperature during the development of larval stages of the metacercariae used in experiments 2, 3 and 5 could explain their lower infectivity. Also, it may be possible that due to longer storage, most of the metacercariae used in these experiments had lost their viability and this was not recognised when they were counted under the low power stereomicroscope.

It was interesting to note that the metacercariae used in experiment 4 were found to be highly infective, despite the temperature in the refrigerator in which these were stored, dropping to below 0° C, possibly for at least 24 hours. This observation suggested that the *F. gigantica* metacercariae produced in natural conditions may have a better ability to withstand the deleterious effects of freezing which were observed by Boray and Enigk (1964) than the metacercariae produced under laboratory conditions; these workers observed that freezing at low temperatures did not kill the metacercariae, but apparently caused irreversible changes which made them unable to infect the hosts.

Despite the use of metacercariae obtained from similar sources and stored under identical conditions, there was a significant difference between the recovery of flukes from sheep (experiment 2), goats (experiments 3) and buffaloes (experiment 5) (F = 5.208, d.f. = 2, P = 0.0136). Although, other factors might have been responsible for this difference, the possibility that these animals had a varying susceptibility to *F. gigantica* infections, cannot be precluded.

9.5 Pathological Observations

The pathological findings of *F. gigantica* infections described by Ogunrinade (1984) and Ajanusi et al. (1988) in sheep; Ogunrinade (1984) and Singh and Parihar (1988) in goats; and Swarup and Pachauri (1987c) and Matto, Bali and Gupta (1989) in buffaloes closely resembled the lesions which were found in the livers in the present study. The early lesions observed in two sheep in experiment 1 and four buffaloes in experiment 4, such as enlarged livers with prominent haemorrhagic tracts and areas of fibrinous peritonitis on the surface together with large quantities of blood and blood-stained fluid in the intestines and peritoneal cavity, are characteristic of the subacute fasciolosis and were similar to those described in experimental *F. gigantica* infections in sheep (Ajanusi et al., 1988) and cattle (Ogunrinade, 1983). The main difference in the liver lesions reported by these authors from that seen in the sheep and also in one buffalo in the present study was the absence of blood clots in the gall bladder. Enlarged gall bladders and distended bile ducts with thickening of the walls were major gross lesions in the chronic phase of the infection.

The histopathological lesions observed in the present study were characterised mainly by periportal fibrosis, infiltration of mononuclear inflammatory cells in periportal areas and bile duct lamina propria and fibrosis and hyperplasia of bile ducts. Infiltration of eosinophils in periportal areas and bile duct lamina propria was markedly evident in sheep and goats, but not in buffaloes which agreed with the findings of Swarup and Pachauri (1987c) and Matto et al. (1989). Increase in the goblet cells of the biliary epithelium seen in buffaloes in the present study was also observed by Matto et al. (1989).

The severity of hepatic lesions observed in infected animals varied between individuals within experimental groups and was related to the number of flukes recovered. Although flukes were not recovered from two of the infected sheep in experiment 2 at necropsy, the livers of both the sheep exhibited gross and microscopical lesions typical of chronic fasciolosis which suggested that the sheep had *Fasciola* infection either before or during the experimental period.

In general, the lesions observed in the three species of domestic ruminants under study were comparable to those observed in cattle by Hammond (1970) and Ogunrinade (1983), except that calcification of the bile ducts was never observed in any section examined in the present study. This observation corroborates the findings of earlier authors (Ogunrinade, 1984; Swarup and Pachauri, 1987c; Ajanusi, et al.,1988; Singh and Parihar, 1988; Matto et al., 1989) in these species. Hammond (1970) observed calcification of bile ducts in one calf infected with *F. gigantica* as early as 15 WPI, while Ogunrinade suggested that this appear to be a late phenomenon in fasciolosis in cattle appearing between 200-365 days post infection.

The widespread pathological lesions observed in buffalo calf 83 in experiment 4 were similar to those described by Janzen et al. (1981) and Orr, Osborne and Janzen (1982) in bacillary haemoglobinuria in cattle. They considered that liver necrosis caused by migrating young fluke (Janzen, et al., 1981) or *Fusobacterium necrophorum*, provide conditions suitable for development of resident clostridial spores in the liver.

9.6 Haematological Observations

9.6.1 *Erythrocytic series*

Except for the sheep in experiment 2, declines in RBC counts, PCVs and haemoglobin concentrations in infected animals were observed consistently. These declines were found to be correlated with the levels of infection which is in agreement with the conclusion of Ogunrinade and Bamgboye (1980). It was surprising, however, that the changes in absolute erythrocytic indices varied from experiment to experiment. For example, in sheep in experiment 1, a macrocytic normochromic anaemia was observed, whereas a normocytic hypochromic anaemia developed in goats in experiment 3, macrocytic hypochromic in buffaloes in experiment 4 and normocytic hypochromic in buffaloes in experiment 5. It must be emphasised, however, that these changes were neither very pronounced nor consistent, except for the buffaloes in experiment 4.

Although, normocytic normochromic anaemia has been reported to be characteristic of fasciolosis by several workers (Sinclair, 1964; Sewell, 1966; Hammond, 1970; Ogunrinade, 1983; Swarup et al. 1987; Chaudhri et al., 1988; Wiedosari and Copeman, 1990), there are also reports of macrocytic hypochromic (Reid, Armour, Urquhart and Jennings, 1970; Haroun, Gadir and Gamel, 1986) and macrocytic normochromic (Mohsin, Rahman, Das and Fazlul Huque, 1991) anaemia. In the present study the rises in MCV and falls in MCHC values were very marked in the buffaloes in experiment 4. Mohsin et al. (1991) suggested that deficiencies of Vitamin B_{12}, folic acid, niacin and cobalt which usually increase MCV values may be associated with fasciolosis. Reid et al. (1970) pointed out that it is important to eliminate factors other than fasciolosis which may alter MCHC values, e.g. nutrition and other helminth parasites. However, although fodder was becoming scarce from mid April (9 WPI) onward, the buffaloes were still on an adequate diet. Similarly, a small nematode burden was recorded in most of the buffaloes, but their numbers were not sufficient to account for the haematological changes which occurred.

9.6.2 *Leucocytic series*

Both, the leucocytosis and the eosinophilia observed in infected animals in the present work, generally followed a similar pattern suggesting that the latter was mainly responsible for the former. However, these changes were not related to the intensity of the infection, which agreed with the findings of Ogunrinade and Bamgboye (1980). From the results of his own experiments and from comparisons with those of other workers, Hammond (1970) concluded that the only clearly defined change occurring in the leucocyte picture in fasciolosis in sheep and cattle is a consistently high eosinophilia; considerable variations may occur in the numbers of lymphocytes and neutrophils, but monocytes and basophils fluctuate within a normal range.

In the present study, eosinophilia in infected animals commenced 2-3 WPI and persisted almost throughout the experimental period, except in buffaloes where it was found to persist only until 24-28 WPI. Also, the levels in buffaloes were not as high as those in sheep and goats. The presence of an eosinophilia in the two infected sheep from which flukes were not recovered, gave a further evidence that the infections in these sheep terminated before necropsy. However, the reason for the additional peak observed at 22 WPI in one of these and at 28 WPI in another, remain unexplained.

Eosinophils are believed to be important effector cells involved in the immune killing of secondary *F. hepatica* infections of rats, operating most effectively on juvenile flukes entering the peritoneal cavity (Davis and Goose, 1981; Burden, Bland, Hammet and Hughes, 1983). It has been suggested that the inferior eosinophilia of mice infected with *F. hepatica*, relative to rats, may contribute to the failure of mice to develop resistance to secondary infection (Milbourne and Howell, 1990). Furthermore, sheep which develop strong resistance to reinfection with *Trichostrongylus columbriformis* were found to exhibit a significantly higher blood eosinophilia than their *T. columbriformis* infected counterparts which offered only weak resistance to reinfection (Dawkins, Windon and Eagleson, 1989). These studies suggest that the magnitude of the eosinophilic response mounted will influence the ability of the host to develop resistance to secondary infection. It can not be precluded, therefore, that the inferior eosinophilia together with the absence of tissue infiltration by eosinophils observed in infected buffaloes in the present study indicate a poor ability of this host to develop resistance to secondary infection.

Immunological reactions, including the development of the eosinophilic response, are modulated by a network of events controlled by lymphokines (Klein, 1990). Eosinophilia is generally believed to be mediated by T-cells (see Section 6.3.2) which produce, among other factors, eosinophil differentiation factor (EFD) which is also known as interleukin-5 (IL-5) (Sanderson, Campbell and Young, 1988). Interleukin-5 has been shown to cause proliferation of eosinophils in bone marrow cultures by stimulating pre-committed eosinophil precursors (Sanderson et al., 1988), and is, itself, thought to be produced following T-cell stimulation with interleukin-2 (IL-2) (Enokihara, Furusawa, Nakakubo, Kajitani, Nagashima, Saito, Shishido, Hitoshi, Takatsu, Noma, Shimizu and Honjo, 1989). The generation of cells committed to the eosinophil lineage appears to result from interleukin-3 stimulation of bone marrow stem cells (Klein, 1990). The eosinophil response may be downregulated by feedback mechanisms acting on lymphokine production. Besedovsky, Del Rey, Sorkin and Dinarello (1986) suggest that following infection, or during inflammatory or immune responses, interleukin-1 concentrations in the circulation may reach a level which stimulates the pituitary-adrenal axis and results in increased glucocorticoid levels in the blood. Glucocorticoids inhibit the production and action of several lymphokines, including IL-2 (Besedovsky et al., 1986). In the present study, the rises in the eosinophil counts were generally biphasic, with the peaks at 3-4 and 12-13 WPI. Hammond (1970) also observed a similar pattern of eosinophilia in his infected sheep and cattle. The fall between the two peaks may be due to either a sudden demand for eosinophils in tissue which has been met by removing these cells from the blood as suggested by Archer (1963), or it may be a consequence of stimulation of the pituitary-adrenal axis.

9.7 Biochemical Observations

9.7.1 *Serum proteins*

Slight rises in total serum protein levels but significant rises in globulin levels, were observed in infected goats 11-16 WPI and in infected buffaloes 7-14 WPI. These determinations were not carried out in sheep in experiment 1, but the rise in serum protein was not evident in sheep in experiment 2 which had very low grade infections. The initial rises in total serum protein and serum globulin concentrations were followed by reductions in the both globulin and albumin fractions of protein, these being more pronounced in serum albumin levels. The changes in the concentrations of total serum protein including globulin and albumin were found to be related to the intensity of the infection.

The initial rises in total serum protein and globulin levels have also been recorded by previous workers in *F. gigantica* infected cattle (Hammond, 1970) and *F. hepatica* infected sheep (Hammond, 1970; Reid et al., 1970). Hammond (1970) showed that much of the increase in serum globulin concentration in infected cattle was due to an increase in the γ-globulin fraction, although there were some increases in the α and β fractions. Reid et al. (1970) stated that the initial elevations in γ-globulin concentrations in fasciolosis are probably the result of two factors; an immunological response to invading metacercariae and a generalised increase in reticulo-endothelial activity associated with liver damage.

The lower serum total protein and albumin levels which were observed in infected animals after 13-16 WPI in the present study, agree with observations by Chaudhri et al. (1988) in *F. gigantica* infections in buffaloes and Reid et al. (1970) in *F. hepatica* infections in sheep. Fall in the serum protein concentration have been shown by Holmes et al. (1968b) to be associated with hypercatabolism of albumin and globulin due to loss of protein into the bile ducts of the infected animals. The liver is the sole source of albumin (Paton, 1969), therefore, the hypoalbuminaemia observed in fasciolosis may also be due to reduced synthesis of albumin in the liver as a result of the damage caused by flukes.

9.7.2 *Serum enzymes*

As early as 2-3 WPI, serum GLDH values were elevated in infected animals, and generally the higher levels persisted until the end of the experiments. In sheep and goats (experiments 2 and 3), the rises in the levels followed a biphasic pattern, with the peaks at 4-5 and 10-11 WPI. However, this was not found in buffaloes, in which a monophasic rise with a peak at 9-11 WPI was observed.

Hughes, Treacher and Harness (1974) suggested that in low grade *F. hepatica* infection in goats, GLDH levels reach a peak at the period of maximum activity of the flukes in the liver parenchyma before they enter the bile ducts, while in heavy infections there is such a large amount of liver damage that this occurs by week 3. However, this suggestion does not explain the reason of declines in the levels in sheep and goats which were observed after the first peak. It must be emphasised here that the GLDH assay kits which were used in the present study, are manufactured for the use in human medicine to assay relatively low levels of enzyme activity. It is possible, therefore, that an inadequate amount of substrate available for the enzyme levels present in the sera resulted in early exhaustion of the reaction giving false lower values, although the sera were diluted according to the manufacturer's instruction.

The increased GLDH levels in serum following *Fasciola* spp. infection may be explained by its distribution in liver tissues and the damage arising from an initial migratory phase of immature flukes in the parenchyma. An increased level of GLDH concentration later in the biliary stages may be due to the effects of fibrosis. Treacher et al. (1974) observed the later secondary increase in GLDH concentration by placing immature *F. hepatica* in the biliary system of goats. These workers suggested that it may be due to diffusion of toxic products or an auto-immune response.

In the present study, GGT levels increased 8 WPI in sheep infected with *F. hepatica*, 10-12 WPI in sheep and goats infected with *F. gigantica* and 7 WPI in buffaloes infected with *F. gigantica*; the levels remained high until the end of the experiments. In *F. hepatica* infections, the maximum levels were observed 10 WPI, while in *F. gigantica* infections these were found to be at 12-17 WPI in sheep. These results correspond to the observations of Anderson et al. (1978), Sykes et al. (1980) and El Samani et al. (1985).

GLDH is considered to be the enzyme of choice in measuring hepatic necrosis in ruminants (Kaneko, 1980). Serum GGT levels in ruminants are little affected by hepatic necrosis, but are useful indicators of the degree of intra- or extrahepatic cholestasis (Kaneko, 1980). Findings of the present study suggested that, although the increased serum GLDH and GGT levels are sensitive indicators of *Fasciola* infections, these are not related with the intensity of infection. Hughes et al. (1973) also found no correlation between numbers of flukes present in individual goats and the GLDH activity in the plasma.

9.7.3 *Serum minerals*

Serum mineral levels were determined only in buffaloes in experiments 4 and 5. Calcium, magnesium, phosphorus and copper levels fell from 8-12 WPI. Although, lower potassium and sodium levels were generally observed in infected buffaloes, the trends were not very definite. The declines in the concentrations of these minerals did not appear to be correlated with fluke burdens.

Haiba et al. (1964) detected a fall in calcium but no changes in magnesium or inorganic phosphate in buffaloes naturally infected with *F. gigantica* while Chaudhri et al. (1988) observed significant decreases in calcium and inorganic phosphate. Sinclair (1962; 1965) detected a fall in calcium and magnesium levels in sheep while Jablonowski, Liminowicz and Romanik (1971) and Dessouky and Moustafa (1978) did not detect changes in either sodium or potassium but did report a fall in organic phosphate levels. Enigk, Feder, Dey Hazra and Weigartner (1972) who found a fall in level of sodium after the second week of infection, stated that the levels of electrolytes depended on the period of infection and its level. Ogunrinade, Adenaike, Fajinmi and Bamgboye (1980), however, could not associate the electrolyte variables with the different levels of *F. gigantica* burden.

9.8 Agar Gel Diffusion Assay

In heavy infections (in buffaloes in experiment 4), precipitin lines were observed in the sera as early as 2-3 WPI, while in light infections (sheep in experiment 2), the lines became evident only after 6 WPI. These lines became fainter from 17-20 WPI and began to disappear from 22 WPI.

Kendall, Sinclair, Everett and Parfitt (1978) also showed that precipitating antibodies appeared in the sera of cattle 2 to 3 weeks after primary infection with *F. hepatica*, rising to a maximum level about the 12th week after infection. These observations together with those in the present study suggested that the adult flukes made little contribution to the stimulation of precipitating antibodies once they reached the main bile ducts. The fainter lines observed in the sera of lightly infected animals in the present study agree with the findings of Sinclair and Kendall (1969) who observed a good correlation between the precipitin titre and the number of flukes recovered from individual rabbits infected with *F. hepatica*.

Sera from 2 buffaloes showed faint precipitin lines during preinfection period. It seems unlikely, however, that these animals were infected with *Fasciola spp.* before the experiment started, because their serum GLDH and GGT values were within the normal range during the preinfection period. Possession of common antigens by various helminths is well known (Van Tiggele and Over, 1976; Burden and Hammet, 1978; Ogunrinade, 1983; Fagbemi and Obarisiagbon, 1991), and buffaloes in the hills of eastern Nepal are commonly found to be infected with other trematodes, such as paramphistomes (Singh et al., 1973) and *E. cladorchis* (Mahato, Harrison and Hammond, unpublished). These false positive results in the agar gel diffusion assay were not therefore totally unexpected especially when whole fluke extract was used as antigen. However, results of the present study showed that the assay is useful, quick and simple for monitoring experimental infection of animals to check for prepatent *F. gigantica* infections.

PART THREE

MOLECULAR BIOLOGY

CHAPTER TEN

LITERATURE REVIEW: DIFFERENTIATION OF *FASCIOLA* SPP.

10.1 Introduction

Attempts to differentiate *Fasciola* spp. have been carried out using morphology, physiology, immunology and biochemistry both alone and in combination; each technique being used to compare a different characteristic of the chosen species. *Fasciola hepatica* and *F. gigantica* have, however, largely been distinguished on the basis of their distinct morphology and specificities for different intermediate hosts. Following a brief survey of the species of *Fasciola* described in the literature, the methods available for their differentiation and the difficulties encountered with them are reviewed in this chapter.

10.2 The valid and doubtful species of Fasciola

Liver flukes are digenetic trematodes belonging to the family *Fasciolidae* Railliet 1895 and genus *Fasciola* Linnaeus 1758. The parasite was first described by Gabucinus in 1547 and Linnaeus gave it the definitive Latin name, *Fasciola hepatica* in 1758. No other species was known until 1855 when Cobbold described *F. gigantica* from the liver of a giraffe from Ethiopia (cited by Hammond, 1970).

Railliet (1895) described *F. hepatica* var *angusta*, and Looss (1896) *F. hepatica* var *aegyptiaca*, but both these varieties were regarded as identical with *F. gigantica* by Blanchard (1896)(cited by Malviya, 1967). Sinistin (1933) identified *F. halli* and *F. californica* as separate species in America on the basis of minor morphological differences in adult flukes. Price (1953) examined *Fasciola* spp. from various parts of the United States and found that while those from some areas appeared to be identical to *F. hepatica* from the old world, others approached *F. gigantica* in shape. A form that seemed intermediate in morphology between the *F. hepatica* and *F. gigantica* also occurred.

Varma (1953) described a new species of liver fluke, following his study of *Fasciola* spp. infections in cattle, buffaloes and goats in Bihar, India and the examination of specimens sent to him from Assam, Rangoon and Singapore. He named it *F. indica* which was differentiated from *F. hepatica* and *F. gigantica* on the basis of cuticular armature, gut branching, and testes of the adult and shape and size of the eggs. Tandon (1956) accepted *F. indica* but Sarwar (1957), Kendall and Parfitt (1959) and Malviya (1967) questioned the validity of this new species and considered *F. indica* to be identical with *F. gigantica*. When Kendall and Parfitt (1959) studied both flukes and eggs from Pakistan and Africa, they were unable to distinguish between *F. gigantica* from Africa and *F. indica* from Pakistan. Similarly, Malviya (1967) found that the large liver fluke found in India is morphologically indistinguishable from *F. gigantica*.

Watanabe (1962) suggested that an intermediate form of fluke existed in Japan that was the same as *F. indica*. However, Oshima, Akahane and Shimazu (1968) stated that the common Japanese liver fluke was a single group within the range of variation presented by *F. hepatica*, *F. gigantica* and *F. indica*.

Huang, Lai, Chen and Shien (1979) considered that the three forms of *Fasciola* spp. in Taiwan represented a single variable species, a conclusion also reached in Japan by Oshima et al. (1968). Watanabe (1965; 1967) carried out an extensive biological and morphological study and concluded that two species of *Fasciola* occur in Japan: *F. gigantica* which is rare and geographically localised, and a Japanese *Fasciola sp.* which is by far the more common.

Zdun and Yavorskii (1984) divided *F. hepatica* into three sub-species such as *F. hepatica oblongata*, *F. hepatica ovata* and *F. hepatica lineata* but Ternopol'skaya (1984) suggested that similar morphological differences to those attributed to the above sub-species occur if the same species of *Fasciola* parasitises different species of mammalian host.

Kendall (1965) considered that *F. hepatica* and *F. gigantica* are probably the only two valid species. It has been suggested that geographical isolation may be responsible for the appearance of various morphological strains (Malek, 1980) and physiological races (Kendall, 1965) of *Fasciola* spp. Despite this suggestion there still exists considerable confusion and much debate regarding the different species of *Fasciola*. Malek (1980), in reviewing the differences between *F. hepatica* and *F. gigantica* considered that there was no simple way of distinguishing them by examination of adults and eggs. However, other species of *Fasciola*, generally considered to be valid in view of their gross morphological differences are *F. nyanzae* which is limited to East Africa where it parasitises hippopotamus, *F. tragelaphi* found in the sitatunga (*Tragelaphus spekei*) in Africa and *F. jacksoni* which is found in elephants in India (Hammond, 1970).

10.3 Morphometric and morphological differences

10.3.1 *Ova*

Morphometric studies of eggs have been used to differentiate between *Fasciola* spp. (Taylor, 1964; Tinar, 1984). Soulsby (1982) refered to *F. hepatica* ova as measuring 130-150 μm in length and 63-90 μm width and *F. gigantica* ova as 156-197 μm in length and 90-104 in width.

Kendall and Parfitt (1959) observed that although there appeared to be no differences in the size of *F. gigantica* eggs collected in Africa and Asia, there was a significant difference between the eggs of *F. gigantica* and those of *F. hepatica*. All eggs examined were freshly isolated from the faeces of animals with mature *Fasciola* infections. However, the proposed species *F. indica* could not be distinguished from *F. gigantica* by comparative studies using egg measurements (Kendall and Parfitt, 1959).

Hammond (1970) suggested a note of caution that egg size and shape may change due to fixation and storage, especially if the storage solutions are not isotonic. He further stated that egg size may be affected by prolonged storage in the gall bladder and by size of the flukes. He also suggested that fully mature eggs may differ from those taken from the uterus of the flukes.

10.3.2 *Rediae*

Ollerenshaw and Graham (1986) claimed that *F. hepatica* and *F. gigantica* can be readily separated on the relative length and shape of the blind intestine in all but the most juvenile of the rediae stage in both preserved and recently killed snails.

10.3.3 *Adult flukes*

Among the features by which *F. gigantica* is differentiated from *F. hepatica* are that it is longer, the shoulders are less developed, the cephalic cone is shorter, and the caeca are more branched especially those towards the midline of the body; the branches of the ovary are longer and more numerous in *F. gigantica* but smaller and club-shaped in *F. hepatica* and the average distance between the posterior border of the body and the posterior testes is longer in *F. gigantica* (Malek, 1980). The two species may also be differentiated on the basis of average length to width ratio which is 4.39-5.20:1 for *F. gigantica* and 1.88-2.32:1 for *F. hepatica* (Sahba, Arfaa, Farahmandian and Jalali, 1972).

In differentiating *Fasciola* spp, much emphasis has been placed on the shape and size of the adult body. However, Jackson (1921) stated in his review of literature on *F. gigantica* that neither shape nor size of the adult body could be used for the differentiation of species because of the distortion of the body inevitably produced by maceration and fixation, unless there were distinct anatomical differences among the species. He therefore performed a comparative study between *F. gigantica* and *F. hepatica* using a large number of fresh specimens of flukes. The study revealed consistent morphological differences in the branching of the caeca and cuticular armature.

The difficulty in morphological differentiation between *Fasciola* spp. has been realised by several workers. According to Taylor (1964) no outstanding feature has been recognised, even in *F. hepatica* and *F. gigantica*, and it is not possible to decide from the general body outline to which species some individuals belong. Kendall (1965) and Boray (1969) pointed out that there is in fact great variation in the size of *F. hepatica* and *F. gigantica* depending on such factors as the age of the flukes, the species of host in which it is found and weight of infection. It was noticed by Hammond (1972) during the survey of *F. gigantica* infection of wild mammals in East Africa that specimens from some species were much larger than from others.

A range of morphological types of *Fasciola* occurs in Southeast Asia (Japan, Taiwan, Philippines). At extremes of this morphological range, early authors described some as resembling *F. hepatica* on the one hand and *F. gigantica* on the other, with an intermediate form also occurring (Kendall, 1965). A recent attempt to clarify the species of liver fluke in naturally infected carabaos in the Philippines by morphological investigations concluded that the specimens examined were akin to *F. hepatica, F. indica* and *F. gigantica*, but that it was difficult to distinguish them purely by their shape and size (Kimura, Shimizu and Kawano, 1984).

10.4 Cytological differences

Studies of *Fasciola* karyotypes have not clarified the speciation situation in Japan. Moriyama, Tsuji and Seto (1979) and Sakaguchi (1980) reported that the Japanese *Fasciola* sp. has 20 or 30 chromosomes, apparently corresponding to diploid and triploid sets. Some worms exhibited a mosaic of ploidy (mixoploid). Spermatogenesis was aberrant and sperm generally absent. Oogenesis was also aberrant with primary oocytes containing 20 or 30 chromosomes. Sakaguchi (1980) concluded that the Japanese *Fasciola* sp. is parthenogenetic. Diploid and triploid forms differ in body size (Sakaguchi, 1980) and egg sizes (Moriyama et al., 1979). It is not clear from the literature whether the various ploidy states correspond with the morphological types recognised in Japan by the earlier workers (Watanabe, 1962; Oshima et al., 1968).

10.5 Biological differences

A requirement of different snail hosts and different development in these have been suggested in support of the factors in difference in *Fasciola* spp. (Hammond, 1970).

Differences in host specificity is also shown in the final hosts. Sewell (1966) stated that *F. gigantica* appears to be better adapted as a parasite of cattle than *F. hepatica*. He noted that the heavy fibrosis and calcification in the livers of cattle infected with *F. hepatica*, was less marked than in those infected with *F. gigantica*. He also pointed out that *F. gigantica* develops relatively poorly in rabbits and other laboratory animals. Mango, Mango and Esamal (1972) showed that rats are refractory to *F. gigantica* infection and although infection occurred in mice, hamsters, guinea-pigs and rabbits, the flukes did not attain sexual maturity as no eggs were excreted by 10-22 weeks after infection. Similar results with mice have been obtained by Boray (1963) and Hanna and Jura (1976). In contrast, mice (Dawes, 1962; Dawes and Hughes, 1964) and rabbits (Urquhart, 1954; Haroun, 1969) have been shown to be invaluable laboratory hosts for *F. hepatica*.

10.6 Serological differentiation

Sewell (1964) reported that *F. gigantica* can be distinguished from *F. hepatica* by use of antisera prepared in rabbits against non-protein somatic antigens, but the sera of naturally infected animals was not species specific. Although, several serological methods have been used for the diagnosis of fasciolosis, serology has not figured greatly in attempts to differentiate between *Fasciola* spp. Indeed, in general, even the attempts to use immunological methods for the diagnosis of fasciolosis have been unsatisfactory because of the possession of common antigens by various helminths (Van Tiggele and Over, 1976; Lehner, 1977; Burden and Hammet, 1978; Fagbemi and Obarisiagbon, 1991). Thus a great deal of the work within the field of immunodiagnosis of *Fasciola* infections have been directed towards the search for species-specific antigens, in the hope that they will facilitate the development of more accurate and reliable methods (Hillyer, 1980; Hillyer and Soler de Galanes, 1991; Fagbemi and Obarisiagbon, 1991).

The enzyme linked immunosorbent assay (ELISA) has been applied as a diagnostic method for fasciolosis (Burden and Hammet, 1978) and may prove useful for studies on the differentiation of species. Antigen for such studies could be isolated from polyacrylamide gels by electroelution (Santiago and Hillyer, 1986), or produced by techniques involving monoclonal antibodies. Monoclonal antibody techniques were recently used to purify antigen from mature *F. hepatica*. This was used to detect antibody to *F. hepatica* in sera from sheep and cattle. Host species differences in response to infection by *F.hepatica* were demonstrated (Trudgett, Anderson and Hanna, 1988). Selected monoclonal antibodies might also prove useful in differentiating between *Fasciola* species.

10.7 Protein-based biochemical methods

10.7.1 *Whole-body proteins*

As a consequence of genetic variation the amino acid composition of specific proteins change. It is therefore possible to assess how closely organisms are related by comparing the structure of their homologous proteins. Proteins possess two physical characteristics, molecular weight and electric charges, which may conveniently be used to separate them. Separation may be carried out by several methods including zone electrophoresis on paper, cellulose, agarose gel, starch gel, or polyacrylamide and also by isoelectric focusing.

Protein extracts from *F. hepatica* and *F. gigantica* collected from livers at a meat packing plant were compared by polyacrylamide disc gel electrophoresis (Klimenko and Sazanov, 1972). There was a marked difference in protein banding patterns between *F. hepatica* and *F. gigantica.* However no such differences were seen between *F. hepatica* and *F. indica* flukes obtained from an experimentally infected sheep. Lee and Zimmerman (1993) compared *F. hepatica* and *F. gigantica* whole-body protein extracts by isoelectric focusing. Many proteins appeared common to both species and some were shared with host tissue, however, the protein profiles of the two species were different. Lee, Zimmerman and Wee (1992), using the same technique, showed that the overall banding pattern of *F. hepatica* from Korea was essentially identical to that of *F. hepatica* from the United States, which provided support for the usefulness of this technique in differentiating *Fasciola* spp. even from different geographical areas.

Electrophoresis and isoelectric focusing of the complex mixtures of proteins present in a metazoan such as *Fasciola* spp. are relatively simple to perform, with reproducible results, but difficult to interpret due to the large numbers of components encountered. Rather than using whole-body protein extracts, less complex mixture of excretory or secretory products could be used as an alternative. However, this will require incubation of the live specimens in culture medium. Furthermore, results from protein-based methods can be influenced by external factors (Hussey, 1979; Nadler, 1990) which may affect gene expression at the transcriptional, translational or even post-translational levels. Thus, information on the level of normal variability within a particular set of alleles becomes a critical parameter for interpreting protein data and determining whether the observed differences can be attributed to intraspecific variation.

10.7.2 *Isoenzymes*

An alternative to comparing all the proteins of an organism is to restrict the study to enzymes. After separation by electrophoresis, multimolecular forms of enzymes (isoenzymes) can be easily identified by use of the appropriate substrates in specific staining systems (Harris and Hopkinson, 1976).

Although the kinetics and electrophoretic properties of many enzyme systems in different stages of *Fasciola* spp. have been studied by various workers (Alcaino, Baker and Fisk, 1976; Probert and Lwin, 1976; 1977; Behm and Bryant, 1982), only a few reports on the application of enzyme electrophoresis for differentiation of *Fasciola* spp. are available. Agatsuma and Suzuki (1980) and Agatsuma (1981) performed electrophoretic investigation on enzymes of the common liver fluke in Japan as a basis for determination of controversial taxonomical status of these flukes.

Mahato (1984) investigated the application of enzyme electrophoresis in thin layer starch gel for differentiation of *Fasciola* spp. He demonstrated differences in the glucose phosphate isomerase (GPI) zymograms of *F. hepatica* and *F. gigantica* but different stages of the same species and different individuals had identical zymograms. Also, he demonstrated that the technique is very effective for identification of *Fasciola* spp. in infected snails as early as 7 and 12 days after infection with *F. hepatica* and *F. gigantica* respectively. Trawford (1988) used isoelectric focusing on ultra thin polyacrylamide gels and confirmed the observation of Mahato (1984) that GPI isoenzymes differ in these two species of *Fasciola*.

Although, enzyme electrophoresis and isoelectric focusing have been found to be efficient tools for the differentiation of *Fasciola* spp., there are a number of complicating factors. Harris and Hopkinson (1976) pointed out that multiple enzyme forms may arise from multiple genetic foci or from post translational changes, such as enzymes being bound to coenzymes. Apparently electrophoretically distinct enzymes may also arise as a result of artifacts produced by isolation procedures or storage. Furthermore, most of the enzymes are extremely heat labile and therefore the samples must be stored at low temperatures for enzymatic activity to be maintained.

10.8 Nucleic Acid Based Methods

A comparison of the structure of DNA isolated from individual species is the most direct and sensitive option of comparing species in the absence of morphological criteria. The molecular basis for DNA diagnosis is to allow labelled single-stranded species or strain-specific DNA sequences, selected from well-characterized reference species, to find and hybridize with homologous DNA from, or in, the unknown isolates of parasites.

There had been many exciting developments in DNA diagnosis. The recent advent of the use in parasitology (De Bruijin, 1988) of *in vitro* amplification by the polymerase chain reaction (PCR) and the use of strain or species-specific synthetic oligonucleotides (Harnett, Chambers, Renz and Parkhouse, 1988) has opened up new possibilities. Enzyme cascade amplification of the signal from non-radioactivity labelled probes has given increased sensitivity (Barker, 1987). The use of repeated sequences (Harrison, Delgado and Parkhouse, 1988a; 1988b) and satellite DNA for hybridization fingerprinting means that individual clones and isolates can be distinguished (Rishi and McManus, 1987). '*In situ*' hybridization with recombinant specific sequence probes identify single *Leishmania* or *Trypanosoma* organisms showing well-preserved morphology on microscope slides (Barker, 1987; Gibson, Dukes and Gashumba, 1988).

Although molecular biology has advanced quite rapidly in recent years, its application to study taxonomy and population biology of *Fasciola* spp. has been limited. Irving and Howell (1981) developed an isolation procedure for preparation and *in vitro* translation of mRNA from *F. hepatica*. Techniques for the extraction of nucleic acids from *F. hepatica* and *Dicrocoelium dendriticum* were compared by Quiles, Lopez-Lopez, Hermoso, Monteoliva and Lopez (1985) and cloning and characterisation of cDNA from *F. hepatica* has been carried out by Zurita, Bieber, Ringold and Mansour (1987). Hillyer and Taylor (1988) showed that antibody with specificity for *F. hepatica* mRNA translation products could be detected in rabbit serum 2 weeks after infection. Zurita, Bieber and Mansour (1989) investigated the molecular basis of egg formation in *F. hepatica* by isolating and characterizing an abundant cDNA from a female genital complex cDNA library.

Molecular approach has been applied for identifying *Fasciola* spp. by Blair and McManus (1989). They prepared restriction enzyme maps of the ribosomal RNA genes (rDNA) of *F. hepatica, F. gigantica* and *Fascioloides magna* using three different probes derived from rDNA of *S. mansoni*. They showed that the restriction maps for all samples of *F. hepatica* were virtually identical. Similarly, those for two individual *F. gigantica* samples from Indonesia and from Malaysia were identical. The restriction map for the Japanese *Fasciola* spp. (only one sample) was identical with that for *F. gigantica*. They did not notice any intraspecific variation in restriction sites which might be due to the use of less homologous schistosome-derived probes.

Recently, Shubkin, White, Abrahamsen, Rognlie and Knapp (1992) have developed an oligonucleotide probe derived from a rRNA sequence for the detection of *F. hepatica* in its intermediate snail host. These authors reported that this probe can detect RNA derived from 5 miracidia and distinguish RNA derived from snails infected with *F. hepatica* from RNA of uninfected snails.

CHAPTER ELEVEN

MATERIALS AND METHODS

11.1 Source of Materials

11.1.1 *Fasciola spp.*

Adult *Fasciola hepatica* specimens obtained from the bile ducts of an experimentally infected sheep at the Centre for Tropical Veterinary Medicine (CTVM), Scotland, UK and adult *F. gigantica* specimens obtained from the bile ducts of a naturally infected cattle slaughtered at Dagoratti abattoir, Kenya were used as the source of DNA for cloning. Adult *F. gigantica* specimens collected from the bile ducts of naturally infected buffaloes in Nepal, and *Fasciola* eggs obtained from the gall bladders of naturally infected animals (host not known) in Malaysia and Zimbabwe were used for extraction of DNA to be used in the slot-blot analysis. *F. gigantica* specimens collected from the bile ducts and the migratory tracts in the liver parenchyma of a sheep experimentally infected at CTVM, with metacercariae obtained from naturally infected *Lymnaea natalensis* in Kenya, were used to study the effect of storage of samples on the quantity and quality of DNA.

11.1.2 *Host*

Host materials used in the study were as follows: (a) bovine kidney stored in liquid nitrogen at CTVM (b) liver of a Scottish Blackface sheep collected at the post mortem room, Royal (Dick) School of Veterinary Studies, Field Station, Edinburgh, (c) heparinised blood collected from the jugular veins of an Anglo-Nubian goat kept at the Veterinary Investigation Centre, Bush Estate, Edinburgh (d) heparinised blood collected from the jugular vein of a calf kept at CTVM (e) liver of a buffalo slaughtered at Hile bazzar, Nepal and (f) *Lymnaea auricularia* race *rufescens* snails maintained in the laboratory at Veterinary Investigation and Analytical Services Section, Pakhribas Agricultural Centre, Nepal.

11.2 Preparation and Storage of Samples and Stock Solutions

11.2.1 *Fasciola specimens and host materials collected in Nepal and Kenya*

The fluke specimens obtained from the bile ducts of naturally infected animals were washed several times in sterile 0.85% (w/v) saline or phosphate buffer saline (PBS), pH 7.3 (Dulbecco A, Oxoid). The specimens were rinsed in 70% v/v ethanol (451015X, BDH) twice, then placed in fresh 70% ethanol in sterile disposable universals (Bibby Sterilin Ltd.). The containers were sealed with parafilm, labelled as appropriate and stored at room temperature until shipment to UK. On arrival at CTVM, the samples were rinsed six times in chilled (4° C) sterile PBS under a sterile hood and stored in liquid nitrogen until DNA extraction.

11.2.2 *Fasciola specimens and host materials collected in Edinburgh*

The freshly collected fluke specimens and host tissues were washed six times in chilled sterile PBS under a sterile hood. After draining the fluid as much as possible, 0.5 ml of the samples were placed in 1 ml plastic capped containers, labelled appropriately and then stored in liquid nitrogen until extraction of DNA.

11.2.3 *F. gigantica eggs from Malaysia and Zimbabwe*

The gall bladders of infected animals were incised and the contents washed with water into a beaker. The *Fasciola* eggs were sedimented in distilled water, with several changes until the supernatant was clear. The eggs were then immersed in 2.12% (w/v) sodium hypochlorite (23039, BDH) solution for exactly five minutes as a condition of importation required by MAFF, UK. The solution was decanted and the eggs washed thoroughly three times with 500 ml sterile distilled water. A bijou bottle was then one-third filled with *Fasciola* eggs and an equal volume of sterile distilled water added so that an air space of approximately one-third of the bijou was left to ensure adequate oxygenation of the eggs. On arrival at CTVM, the eggs were placed in light proof containers and refrigerated at 4°C before being stored in liquid nitrogen. The eggs obtained from Malaysia and Zimbabwe were kept at 4°C for four years at CTVM.

11.2.4 *Bovine and caprine lymphocytes*

Blood was collected from the jugular vein using vacutainers containing lithium heparin as anti-coagulant. Ten ml of blood was diluted with an equal volume of PBS. An aliquot of 4 ml of diluted blood was layered onto 3 ml of Ficoll-Paque (Pharmacia LKB Biotechnology Inc.) in a centrifuge tube of 13 mm internal diameter and centrifuged at 400 g for 30 minutes. The plasma layer was pipetted off leaving the lymphocytes undisturbed at the interface. Using a sterile pipette, the lymphocyte layers from all the tubes were pooled to a clean 50 ml centrifuge tube. The lymphocytes were resuspended in 12 mls of PBS by gently drawing them in and out of a pasteur pipette and then centrifuged at 100 g for 10 minutes. After discarding the supernatant, the fresh lymphocytes were processed further for DNA extraction.

11.2.5 *Storage of fluke specimens in 70% ethanol at 4°C followed by liquid nitrogen*

Freshly collected *F. gigantica* specimens were washed in PBS as described earlier in section 11.2.2. Ten specimens, placed individually in plastic capped containers, were stored in liquid nitrogen on the same day. Remaining specimens were stored in 70% ethanol at 4° C. At monthly intervals for five months, 10 specimens were removed from 70% ethanol, washed six times in PBS and then stored individually in liquid nitrogen until extraction of DNA.

11.2.6 *Stock solutions*

All the reagents used in this study were prepared in ultra-pure water (Reverse osmosis, Millipore System) using molecular biology grades of chemicals. Disposable gloves were worn during preparation and handling of the reagents and buffer solutions. Sterile glasswares and sterile plastic or polypropylene disposables (containers, centrifuge tubes, pipette tips etc.) were used. Stock solutions were sterilized and stored at appropriate temperature as per the method described by Sambrook, Fritsch and Maniatis (1989a).

11.3 Isolation of *Fasciola* spp. and Host DNA

11.3.1 *Equilibrium centrifugation in cesium chloride*

Each fluke was processed individually to obtain its DNA, however, approximately 0.5 to 1 ml of frozen material was used in the case of *Fasciola* eggs and host tissues. First of all, a sterilized mortar and pestle was cooled in an insulated box containing dry ice. The specimens were taken out from liquid nitrogen, removed from the plastic vials and placed in the mortar kept in the box of dry ice. Frozen specimens were then ground into a fine powder using the similarly chilled pestle.

The powder was quickly transferred into a sterile plastic tube containing 7.5 ml of 4% w/v sarcosyl (L-5000, Sigma), 10 mM Tris (T-1503, Sigma)/HCl pH 8.0, 10 mM EDTA (E-5134) pH 8.0 per 1 ml of powdered material. Then 5 mg of Proteinase K (745 723, Boehringer) was added to 7.5 ml of this solution and incubated in a water bath at 37° C for 2 hours. After incubation, solid $CsCl_2$ (C-4036, Sigma) was added at a rate of 0.95 g per ml of solution so that final density (*p*) of the solution was 1.47 g/ml. 200 μl of ethidium bromide (E-8751, Sigma) solution (10 mg/ml) was added to 10 ml of this solution. The mixture was transferred to an ultracentrifuge tube (Quick-seal, Beckman). The tube was filled with $CsCl_2$ solution made up to the same concentration as the mixture and then sealed with an electric heat sealer (P.No. 347646, Beckman). The mixture was centrifuged in an ultracentrifuge using a Type 65 rotor (Beckman) at 150,000 g for 36-48 hours at 22° C. The DNA band was removed from the centrifuge tube using a disposable syringe fitted with an 18 gauge needle under ultraviolet light of 300 nm wave length as described by Sambrook, Fritsch and Maniatis (1989b).

The $CsCl_2$ was removed from the DNA solution by dialysis. The dialysis tubing (D-9277, Sigma) was prepared according to Sambrook et al. (1989a). The DNA solution was transferred into dialysis tubing using a sterile disposable syringe fitted with an 18-gauge needle. The tubing containing DNA solution was placed in sterile TE buffer (pH 8.0) for 3 days at 4° C, changing the buffer twice daily. The DNA solution was transferred in Eppendorf tubes in aliquots and stored at 4° C.

11.3.2 *Phenol/chloroform extraction*

The method was adapted from Sambrook et al. (1989a). Frozen specimens were ground into a powder as described in section 11.3.1. The powdered material was transferred little by little to a 50 ml centrifuge tube containing approximately 10 volumes of extraction buffer [10 mM Tris (T-1503, Sigma)/HCl pH 8.0, 0.1 M EDTA (E-5134, Sigma) pH 8.0 and 0.5% w/v SDS (L-4509, Sigma)]. The tube was shaken to submerge the material in the extraction buffer and then incubated for 1 hour at 37° C in a water bath. To this viscous solution, proteinase K (stock solution 20 mg/ml in water) was added to a final concentration of 100 μg/ml and mixed gently using a sterile glass rod. The suspension was placed in a water bath for 3 hours at 50° C.

The tube was left at room temperature to cool to room temperature, when an equal volume of phenol (P-1037, Sigma) equilibrated with 0.5 M Tris/HCl (pH 8.0) was added. The two phases were gently mixed by inverting the tubes many times until a complete emulsion was formed. The two phases were separated by centrifugation at 3,000 g for 15 minutes at room temperature. The viscous aqueous phase was taken out carefully with a pipette fitted with a wide-bore tip and then transferred to a clean centrifuge tube. The extraction with phenol was repeated twice. The aqueous phase was extracted once with a 50:50 mixture of equilibrated phenol and chloroform (451135V, BDH) and twice with an equal volume of chloroform alone.

Sodium acetate (S-2889, Sigma) was added from a 3 M stock solution (pH 7.0) to a final concentration of 0.3 M to the aqueous phase and mixed well. To this solution, 2 volumes of ethanol (451015X, BDH) was added and again mixed. The solution was stored at -20° C for 30 minutes. The precipitated DNA was removed from the solution either using a hook made from a pasteur pipette and transferred to a eppendorf tube containing 70% ethanol, or recovered by centrifugation at 5,000 g for 5 minutes at room temperature. The DNA pellet was washed twice with 70% ethanol and collected by centrifugation as described above.

As much ethanol as possible was removed by pipette and the tube was left open at room temperature to allow the last visible traces of ethanol to evaporated. The DNA pellet was redissolved in an appropriate volume of either TE (pH 8.0) or pure distilled water. The DNA solution was stored at 4° C.

11.3.3 *Modification of phenol/chloroform extraction for the isolation of DNA from lymphocytes*

The lymphocytes were resuspended in 10 ml of SE buffer (75 mM NaCl, 2 mM EDTA). To this suspension, 50µl of proteinase K (20 mg/ml stock in SE buffer) and 2 ml of 10% SDS were added. After mixing well by inversion, the solution was incubated overnight at 37° C.

Next day, 5 ml of equilibrated phenol and 5 ml of chloroform-isoamyl alcohol (24:1) were added to the solution, and mixed well by repeated inversion over a period of 15 minutes. The emulsion was centrifuged at 3,000 g for 10 minutes. The aqueous phase was transferred to a fresh 50 ml centrifuge tube using a wide bore pipette. The DNA in the aqueous phase was precipitated by mixing 1/30 volume (400 µl) of 2.5 M sodium acetate (pH 5.2) and 1 volume (12.5 ml) of isopropanol. The thread like DNA precipitate was hooked with a sealed pasteur pipette and left in 5 ml aliquot of 70% ethanol for 5 minutes. The DNA was again hooked from the ethanol and allowed to air dry for 5 minutes. The DNA was dissolved in 1 ml TE buffer in an eppendorf tube and stored at 4° C.

11.3.4 *CTAB precipitation*

The DNA extraction was conducted following the method of Yap and Thompson (1987). *Fasciola* specimens were homogenized using a 2.5 ml glass homogeniser (Uniform, Jencons). Approximately 0.5 ml of homogenized material was suspended in 1 ml of lysis buffer [8% v/v Triton X-100 (T-6878, Sigma), 0.25 M sucrose (10274, BDH), 50 mM Tris/HCl, 50 mM EDTA (E-5134, Sigma), pH 7.5] containing freshly prepared proteinase K (745723, Boehringer,) to a concentration of 1 mg/ml. The suspension was incubated at 65° C for 2.5 hours. DNA was precipitated by adding 1 ml of a sterile 2% cetyltrimethyl-ammonium bromide (CTAB) (10391, BDH) to the lysate. A white precipitate containing DNA was pelleted by centrifugation at 1,500 g. After discarding the supernatant, the pellet was dissolved in 0.5 ml of 2.5 M NaCl, 10 mM EDTA, pH 7.7 and diluted with 1 ml of 40 mM Tris/HCl, 2 mM EDTA, pH 7.7. Two volumes of chloroform were added to the solution, mixed gently and then centrifuged at 12,000 g for 5 minutes. Two volumes of ethanol (room temperature) was added to the recovered aqueous phase. DNA was recovered by centrifugation at 12,000 g for 10 minutes, at room temperature and then allowed to dissolve in 1 ml TE buffer by leaving it at 4°C overnight.

11.4 Quantitation of DNA

Determination of the amount of DNA in a sample and an estimate of its purity was carried out spectrophotometrically. Five µl of DNA sample was diluted in 995 µl of TE buffer or pure distilled water.

Each diluted sample was placed in a quartz glass precision-cell (light path 10 mm) (Hellma). The absorbance readings at the wave lengths of 260 nm and 280 nm for each sample was taken in a spectrophotometer (PU 8600 UV/VIS, Pye Unicam) using the diluent as blank. The amount of DNA in the sample was calculated using the method described by Sambrook et al. (1989a) as follows:

OD_{260} of 50 µg DNA/ml at 1 cm light path = 1

Therefore amount of DNA in the sample (µg/ml)

= OD of the sample at 260nm x 50 x 200 (dilution of the sample)

The DNA preparations having OD_{260}/OD_{280} value of about 1.8 were considered pure whereas the preparations having significantly less than this value were considered contaminated with protein or carbohydrates.

11.5 Electrophoresis of DNA

The apparatus used was either Mini-sub (170-4307, Bio-Rad) or Wide Mini-sub DNA (170-4343, Bio-Rad) Electrophoresis Cells. The ends of the ultra-violet transparent plastic gel tray were sealed securely with adhesive tape. The gel tray was levelled on a levelling table. The well comb was positioned on the walls of the gel tray. With the aid of the thumb screws in the holder, the comb was adjusted so that it remained 1 mm above the base of the gel tray.

To make 1% agarose gel, 0.75 g of agarose (A-6013 or A9539, Sigma) and 1.5 ml of X50 TAE buffer were poured into a 250 ml conical flask. The total volume was made to 75 ml with pure distilled water and the flask was swirled to suspend the agarose powder in the liquid. The level of the liquid in the flask was marked with a magic marker. The suspension was boiled in a microwave oven until all the small transparent particles were dissolved. During boiling, the oven was stopped every 40-50 seconds and the flask was swirled gently to maintain a suspension of undissolved agarose. The melted solution was topped up with pure distilled water to make up the total volume of 75 ml. When the molten agarose had cooled down to just about 65°C, 7.5 μl of ethidium bromide (stock 10 mg/ml in water) was added and mixed quickly by swirling the agarose round. The molten agarose was poured into the gel tray quickly.

The gel was allowed to set at room temperature for approximately 1 hour. The comb was carefully removed from the edges of the gel tray. After placing the gel tray onto the DNA cell, freshly prepared gel buffer (12.5 ml x50 TAE, 62.5 μl of stock ethidium bromide, 612.5 ml pure distilled water) was poured to submerge the gel (no more than 4 mm above the surface of the gel).

To adjust the DNA concentrations, the samples were diluted either with TE or pure distilled water. For every 10 μl of the sample, 2 μl of x6 gel loading buffer [0.25% w/v bromphenol blue (B-6896, Sigma), 0.25% w/v xylene cyanol FF (X-4126, Sigma), 40% w/v sucrose (10274, BDH) in water] was added and mixed gently. The samples were loaded to the wells using a micropipette fitted with a fine yellow tip. Then the safety lid was placed on the cell and connected with a power pack (Model 1000/500 constant voltage power supply, Bio-Rad). The gel was run at 60 volts for 45 minutes to 1 hour.

After completion of the electrophoresis, the gel tray was removed from the cell. The excess of gel buffer from the gel was drained out in the cell and the bottom of the gel tray was wiped with tissue paper. The gel tray was placed on a ultraviolet (300 nm wave length) transilluminator (T-2202, Sigma). Photographs of the gel were taken using a polaroid camera (MP4, Polaroid corporation, USA) loaded with high speed black and white polaroid print, Type 57 (F-4513, Sigma) or negative and print, Type 55 (F-4388, Sigma).

11.6 Digestion of *Fasciola* DNA with $EcoR_1$

11.6.1 *Digestion of DNA with $EcoR_1$ under conventional conditions*

11.6.1.1 Digestion 1

The volumes of each sample containing 5 μg DNA were placed in Eppendorf tubes in triplicate and labelled appropriately. To each tube, 2 μl of spermidine (S-2501, Sigma) was added to a final concentration of 4 mM in the reaction mixture. Five μl of x10 high salt buffer (Boehringer) was added to each tube. Then the volume in each tube was adjusted with pure distilled water in such a way that later when the enzyme was added to it, the final volume of each reaction mixture was 50 μl. To the first, second and third tubes, the enzyme, $EcoR_1$ (Cat. No. 703737, Boehringer) was added at the concentration of 5.0, 2.5 and 0.0 unit/μg of DNA respectively. The solution was stirred to mix well and then placed in a water bath at 37°C. After 1 hour of incubation, 25 μl reaction mixture was removed from each tube, inactivated at 65°C for 10 minutes and stored at 4°C. Incubation of the remaining 25 μl of reaction mixture was continued for further 1 hour.

11.6.1.2 Digestion 2

This digestion was designed to use a lower enzyme concentration but longer incubation period. The reaction mixture was set up in 2 tubes for each sample, each tube containing 25 μg DNA. Ultra pure water was added to the samples so that in such a volume that the final volume of reaction mixture (after addition of spermidine, x10 high salt buffer and enzyme) was 100 μl. Then 4 μl of spermidine (to a final concentration of 4 mM) and 10 μl of x10 high salt buffer were added to the sample. The solution was mixed, inactivated at 65°C for 10 minutes, briefly centrifuged and then cooled on ice.

The first tube of each sample was used as control and therefore enzyme was not added to it. Enzyme was added to the second tubes of the each sample at a concentration of 1 unit/μg of DNA. The solution was stirred to mix well and then placed in a water bath at 37°C. At 1st, 2nd, 4th and 6th hours of incubation, an aliquot of 20 μl was removed from the reaction mixture, inactivated at 65°C for 10 minutes and stored at 4°C. The remaining 20 μl of reaction mixture was incubated for 22 hours, then inactivated and cooled on ice.

11.6.2 *Digestion under star conditions*

The volume of DNA sample containing 15 μg DNA was placed in a tube. Five μl of x10 medium salt buffer (Boehringer) was added to the tube. The volume was adjusted with ultra pure water so that the volume of the reaction mixture after addition of the enzyme was 50 μl. The nucleotides in the sample were inactivated by incubating the solution at 65°C for 10 minutes. The solution was briefly centrifuged and cooled on ice. To the solution, 225 Units of the enzyme $EcoR_1$ (15 units/μg of DNA) was added. The solution was stirred to mix well and incubated at 37°C in a water bath.

After 4 hours of incubation, 16.66 μl (5 μg DNA) of the digest was removed, the enzyme was inactivated at 65°C for 10 minutes and then stored at 4°C. To the remaining reaction mixture, 150 units of the enzyme $EcoR_1$ (15 units/μg DNA) was added and the incubation was continued for further 2 hours. Again, 17.92 μl (5 μg DNA) of the digest was removed, inactivated and stored at 4°C. The incubation of the remaining reaction mixture was continued overnight (for a total of 22 hours) after addition of 75 units of $EcoR_1$ (15 units/μg of DNA).

11.7 Size Selection of *Fasciola* spp. DNA

11.7.1 *Preparatory gel electrophoresis*

The samples of *Fasciola* DNA, partially digested under conventional and star conditions were pooled separately. The pooled samples were loaded on the preparatory gel which was prepared using a 2 well comb containing 2 smaller outside wells. The outside slots on the gel were loaded with the DNA markers Hind III digested Lambda DNA (D 9780, Sigma). The gel was run at 60V for 1 hour.

The parts of the gel containing 0.5-1, 1-2 and 2-4 kb (kilo base) fragments of the digested DNA were excised into slices using a scalpel blade under ultraviolet illumination of 300 nm wave length. The slice was wrapped in cling film, kept in sterile plastic bijou separately and then stored at 4°C until the DNA was eluted.

11.7.2 *Elution of DNA from agarose gel*

The DNA was eluted from the agarose gel slices, using an electro-eluter (model 422, Bio-Rad Laboratories). The electro-eluter was assembled according to the manufacturers instruction. The glass tubes of the electro-eluter were filled with elution buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA pH 8.0). The gel slice cut into small pieces was placed into the bottom of the tubes. After placing the entire module into the buffer chamber, the lower and upper chambers were filled with approximately 600 ml and 100 ml of elution buffer respectively. The elution was done at constant current of ~15 mA per glass tube for 1 hour. The eluted DNA was collected from the membrane cap using a micropipette.

11.7.3 *Purification of eluted DNA*

One tenth volume of 3 M sodium acetate (pH 7.0) was added to the sample of eluted DNA. Two volumes of ice cold ethanol was added and then mixed well. The samples were left on dry ice overnight. The precipitated DNA was recovered from the solution by centrifugation at 15000 g for 15 minutes at 4° C. One ml of 70% ice cold ethanol was added to each tube and centrifuged at 15,000 g for 5 minutes. The supernatant was decanted and after recentrifugation for 1 minute, the traces of ethanol was pipetted out. The DNA in the tube was resuspended in 10 μl of ultra pure water by allowing the pellet to dissolve overnight at 4° C.

The amount of the DNA fragments in the sample was determined spectrophotometrically and the size was checked by agar gel electrophoresis.

11.8 Construction of *Fasciola* spp. DNA Libraries

11.8.1 *Ligation of Fasciola DNA fragments to Lambda arms*

A DNA Ligation kit (#203003, Stratagene) and Predigested Lamdba Zap II/EcoR$_1$ cloning kit (#236211, Stratagene, USA) were used for ligation of *Fasciola* spp. DNA fragments with Lambda arms. The protocols supplied with the kits were followed exactly.

The amount of *Fasciola* spp. DNA fragments for ligation in approximately equal vector to insert molar ratio was calculated considering 1 kb size of the 0.5 - 1 kb fragments, 2 kb size of the 1-2 kb fragments, 4 kb size of the 2-4 kb fragments and 40 kb size of the vector DNA. One μl (1 μg DNA) of Lambda Zap II/$EcoR_1$ predigested arms was placed in each of 13 tubes. Then the insert DNA was added to the tubes as shown below in Table 11.1.

Table 11.1. Quantity of *Fasciola* spp. DNA fragments used for ligation with Lambda arms.

Sample	Size of insert	Digestion condition	Quantity of DNA
1	*P* Rheo/Ecor1 test insert	not indicated	0.8 μl (≡ 0.4 μg)
2	0.5-1.0 kb *F. hepatica*	star	2.0 μl (≡ 0.025 μg)
3	0.5-1.0 kb *F. hepatica*	conventional	2.0 μl (≡ 0.025 μg)
4	1.0-2.0 kb *F. hepatica*	star	2.0 μl (≡ 0.05 μg)
5	1.0-2.0 kb *F. hepatica*	conventional	0.5 μl (≡ 0.05 μg)
6	2.0-4.0 kb *F. hepatica*	star	1.0 μl (≡ 0.1 μg)
7	2.0-4.0 kb *F. hepatica*	conventional	1.0 μl (≡ 0.1 μg)
8	0.5-1.0 kb *F. gigantica*	star	1.5 μl (≡ 0.025 μg)
9	0.5-1.0 kb *F. gigantica*	conventional	2.0 μl (≡ 0.025 μg)
10	1.0-2.0 kb *F. gigantica*	star	2.0 μl (≡ 0.05 μg)
11	1.0-2.0 kb *F. gigantca*	conventional	0.6 μl (≡ 0.05 μg)
12	2.0-4.0 kb *F. gigantica*	star	1.0 μl (≡ 0.1 μg)
13	2.0-4.0 kb *F. gigantica*	conventional	1.5 μl (≡ 0.1 μg)

To the each tube, 0.5 μl each of x 10 ligation buffer, 10 mM ATP and T4DNA ligase (4U/μl) was added. Ultra pure water was added to the tubes to make the final volume of the ligation solution to 5 μl. The solution was mixed well and incubated at 4° C overnight.

11.8.2 *Packaging recombinant Lambda phage*

Gigapack II Gold Packaging Extract (#200216, Stratagene, USA) was used for packaging the recombinant Lambda phage. Appropriate numbers of the sets of extracts were removed from -70° C freezer and placed on dry ice. At the same time thawing of the sonic extract was started. The Freeze/Thaw extract was quickly thawed between fingers until it was **just beginning** to thaw. **Immediately**, 2 μl of the ligated DNA was added to the Freeze/Thaw extract and placed on ice (4° C). Quickly 15 μl sonic extract was added to the Freeze/Thaw extract containing DNA. The solution was gently mixed with the end of the pipette tip, centrifuged briefly at 13,000 g and incubated at room temperature (22° C) for 2 hours. Then 500 μl of SM buffer (5.8 g NaCl, 2.0 g $MgSO_4$, 50 ml 1 M Tris/HCl pH 7.5 and 5 ml 2% gelatin per litre) and 2.0 μl of chloroform were added to the packaged phase solution and mixed gently. The solution was centrifuged briefly at 13,000 g to sediment debris and then stored at 4° C.

11.8.3 *Preparation of bacterial media*

All bacterial media used were prepared in accordance with Sambrook et al. (1989a)

11.8.4 *Preparation of host bacteria*

The host bacteria, XL1-Blue strain of *Escherichia coli* supplied in a NZY agar stab (#236211, Stratagene) was streaked onto LB Tetracycline plates and incubated overnight at 37° C. A single colony was taken from the plate and grown in 10 ml LB medium supplemented with 0.1 ml 1 M $MgSO_4$ and 0.1 ml 20% w/v maltose (M-5885, Sigma) at 30° C overnight, shaking at 200 rpm. The culture was centrifuged at 2000 g for 10 minutes. The supernatant was discarded and the bacterial pellet was resuspended in 5 ml of sterile 10 mM $MgSO_4$. Before being used, the bacterial suspension was diluted to an OD_{600} of 2.0 with sterile 10 mM $MgSO_4$ (1.5 x 10^9 cells/ml).

11.8.5 *Determination of the phage titres of the libraries*

A ten fold serial dilution from 10^{-1} to 10^{-4} of the packaged phage from the each library was prepared in SM buffer. An aliquot of 10 μl of each dilution of the phage was diluted with 90 μl of SM buffer in a plastic universal. The phage solution was pre-incubated for 20 minutes with 200 μl of the host bacteria, XL1-Blue suspension (OD_{600}=2.0) at 37° C. Three ml of molten NZY top agar supplemented with $MgSo_4$ and kept at 55° C in a water bath was added to the pre-incubated suspension. The suspension was swirled quickly and plated immediately onto the levelled NZY agar plates which were pre-warmed at 37° C for at least 1 hour. The top agar was quickly distributed evenly across the surface and allowed to solidify on a levelling table at room temperature. The plates were incubated upside down at 37°C overnight. The plaques were counted to determine pfu (plaque forming units) per ml,

11.8.6 *Determination of the ratio of recombinants to non-recombinants in the libraries*

An aliquot of the phage suspension was diluted with SM buffer to contain approximately 100 pfu per 10 µl. To the 10 µl of the diluted phage, 90 µl of SM buffer and 200 µl of XL1-Blue host bacteria suspension (OD_{600} =2.0) was added. The suspension was gently swirled and pre-incubated for 20 minutes at 37°C. Then 3 ml of top agar (NZY+$MgSO_4$) and 15 µl of O.5 M IPTG (in H_2O) plus 50 µl of 250 mg/ml X-gal (5-Bromo-4-chloro-3-indolyl-β-D-galactoside) in DMF (dimethylformamide) were added to the pre-incubated vector-host suspension and immediately plated onto pre-heated NZY agar plates. The plates were incubated at 37°C overnight. Next day, the blue and white plaques were counted and the ratios of recombinants (white) to non-recombinants (blue) in the libraries were calculated.

11.9 Screening of the Libraries

11.9.1 *Preparation of plaque lifts*

An aliquot of the phage containing approximately 150-200 pfu was plated out as previously described. After incubation at 37°C overnight, the plaques in the bacterial lawn were counted, the plate was wrapped in cling film and then chilled at 4°C for at least 2 hours to allow the top agarose to harden.

The petri dish was labelled and marked with a magic marker. Similarly the nitrocellulose filter (Schleicher & Schuel) was labelled and marked with a biro and then placed onto the surface of the top agarose aligning the mark on the petri dish. Using a 18 gauge needle, the orientation of the filter in relation to the plate was marked by piercing the filter in several places. The nitrocellulose filter was allowed to wet, then immediately removed gently and placed DNA side upward on a 3 mm filter paper (Whatman) until ready for the next step. Four replica lifts were taken from each plate. For replica lifts, the holes in the agar were relocated and identical holes were punched. The soaking time allowed for the second, third and fourth replica lifts on the top agarose were 15, 30 and 60 seconds respectively.

11.9.2 *Preparation of dot-blots*

Dot-blots of parasite and host genomic DNA were used as controls in the hybridisations for screening the Lambda ZAP lifts. The genomic DNA extracted from *F. hepatica*, *F. gigantica*, bovine kidney and caprine leucocytes were diluted in ultra pure water to contain 250 ng/μl to 0.08 ng/μl. The DNA in the dilutions were sheared by passing the solution through a 23-gauge needle several time. 2.5 μl of each dilution was placed onto a nitrocellulose filter so that the blot contained 625 to 0.2 ng DNA per spot. One spot contained pure water only. The spots were circled around using a biro. The set of 4 dot-blots prepared in this way were air dried and then processed for further steps along with the previously made Lambda ZAP lifts.

11.9.3 *Denaturation and immobilisation of DNA on the lifts/blots*

The plaque lifts and genomic DNA dot-blots were placed in freshly made denaturing solution (0.5 M NaOH, 1.5 M NaCl) for 5 minutes. The filters were transferred to neutralizing solution (1.5 M NaCl, 0.5 M Tris/HCl, pH 8.0) and left in it for 5 minutes. Finally, the filters were rinsed in x 2 SSC, 0.2 M Tris/HCl, pH 7.5 for 2 minutes air dried on 3 mm filter paper (Whatman) for 30 minutes at room temperature and baked at 80° C for 2 hours. The baked filters were placed in an envelope and stored at room temperature until required.

11.9.4 *Labelling DNA with ^{32}P*

F. hepatica, *F. gigantica*, bovine and caprine genomic DNAs labelled with ^{32}P were used as probes for screening of the plaque lifts. The DNAs were labelled with [^{32}P] dCTP (Amersham Ltd) by nick translation (Rigby, Dieckmann, Rhodes and Berg, 1977) using Nick Translation Kit (976776, Boehringer).

The DNAs were diluted in ultra pure water to contain 0.1 μg DNA per 2 μl. The labelling reaction was set in an Eppendorf tubes on ice as follows:

genomic DNA	-	2 μl
Mixture of dATP, dGTP and dTTP (1+1+1)	-	3 μl
Pure H_2O (to final volume of 20 μl)	-	9 μl
x10 Nick translation buffer	-	2 μl
20 μCi [α^{32}P] dCTP, 3000 Ci/mM	-	2 μl
Enzyme mixture	-	2 μl

The reaction mixture was incubated at 15°C for 35 minutes. The reaction was stopped by heating to 65°C for 10 minutes. Care was taken to minimise operator exposure to radiation by using perspex shield and strictly following local rules for working with radio isotopes. The disposables contaminated with radio active material were discarded appropriately and in accordance with local rules.

The non-incorporated deoxyribonucleoside triphosphates were removed by centrifugation in a Centri-Sep Column (Princeton Separation Inc.). The gel in the column was swelled by adding 750 μl of TNE buffer (10 mM Tris/HCl pH 7.8, 0.1 M NaCl, 1 mM EDTA) at room temperature for 30 minutes. The reconstituted columns were inserted into the wash tube provided and centrifuged for 5 minutes in a Biofuge. Then 30 μl of TNE buffer was added to each nick translated probe and applied directly onto the gel bed without disturbing the surface. The columns placed in the sample collection tubes were stoppered and centrifuged for 8 minutes. The columns were removed from the sample collection tube, discarded appropriately (solid radio-active waste).

An aliquot of 1 μl of each purified probe in duplicate was placed in the scintillation bottles. The activity of the probe was measured in a scintillation counter (2000 CA, Tri-carb Liquid Scintillation Analyzer, United Technologies, Packard) using the Protocol £:14, 32P Cerenkovski to determine dpm (disintegration per minute) per μg.

11.9.5 Hybridization procedures

500 ml of freshly prepared pre-hybridization fluid (40 ml x50 Denhardts solution, 100 ml x20 SSC, 5 ml 10% SDS, pure dd H_2O to 500 ml) was poured into

a sandwich box and placed in a water bath at 65°C. After 15 minutes, 1 ml of sheared salmon sperm DNA (10 mg/ml) pre-heated at 100°C for 5 minutes was added to the pre-hybridization fluid. The filters (plaque lifts and dot-blots) were placed in the fluid and incubated at 65°C for 3 hours.

The filters were removed from the pre-hybridization fluid and each replica was placed in separate hybridization bag (8278 BA, Bethestha Research Lab). 30 ml of the pre-hybridization fluid was added to each bag. 50 μl of sheared salmon sperm DNA (10mg/ml) was added to each radio labelled probe and then boiled for 5 minutes in a water bath. The probe was added to the bag containing the filters. The bag was next sealed and then incubated flat overnight at 65°C in a shaking water bath at 50 rpm.

Next morning, the bag was opened and the filters were replaced into 500 ml of the wash buffer (10 ml x20 SSC, 10 ml 10% SDS, pure water to 2 litres) pre-warmed to 54°C. After discarding the radioactive materials appropriately, the wash buffer was poured out almost immediately and a further 500 ml was added. The filters were washed 2 times for 30 minutes in 500 ml of wash buffer at 54°C in a shaking water bath at 150 rpm. After the final wash of 1 hour at the same conditions, the filters were air dried on the 3 mm Whatman filter paper.

11.9.6 *Autoradiography of the probed lifts/blots*

Replica lifts each probed with the 4 different genomic DNA probes were arranged on a sheet of 3 mm filter paper cut to the size of X-ray film. The lifts were attached to the sheet using photographic adhesive dot labels. The sheet was marked with radio-active ink (made by mixing a small amount of ^{32}P with blue fountain pen ink) to several asymmetric locations which later served to align the autoradiograph with the filters. The ink on the sheet was allowed to air dry.

In a dark room, the filter paper sheet was placed in a light-tight X-ray film holder with an intensifying screen and covered with a X-ray film (RP1-Agfa Curix, Agfa). The film was exposed at -70°C for 72 to 96 hours. The film was then removed from the holder in dark room as quickly as possible and immersed in Kodak D-19 developer (P-5670, Sigma) for 5-7 minutes. After rinsing in water for 1 minute, the film was placed in Kodak fixer (P-6557, Sigma) for 5 minutes. Finally, the film was washed under running water for 15 minutes, air dried and then examined for evidence of positive plaques.

11.9.7 *Picking plaques*

When positive hybridization signals were found on the autoradiographs, the positive plaques were identified and picked as described below.

First, the autoradiograph was aligned with the filters using the radio-active ink marks and then marked with the positions of the asymmetrically located needle punch holes in the filters using a fibre-tip pen. The positive plaques were identified by aligning the needle punch marks on the autoradiograph with those on the agar plates.

To pick the plaques, the narrow end of a sterile pasteur pipette was cut and discarded and a rubber bulb was fitted to the cut end. Using this pipette, the positive plaque was stabbed through into the hard agar beneath and a mild suction was applied so that the plaque, together with the underlying agar, was drawn into the pipette. The agar plug was placed in 500 ml of SM buffer containing a drop of chloroform in an Eppendorf tube. The tube was left at room temperature for 1 hour to allow the bacteriophage particles to diffuse out of the agar, and then stored at 4°C.

11.9.8 *Cloning of the picked plaques*

The plaques which were considered either to be strongly cross-reactive or strongly species-specific were cloned for purification. While plating the phages for further screening, an attempt was made to obtain well-isolated plaques on the plates (50-80 pfu/plate). Usually, pure clones of the picked plaques were obtained on the second screening, however, further recloning was continued until a 100% positive hybridization signal was achieved. A total of 5 plaques of each of these pure clones were picked as described earlier, labelled appropriately and then stored at 4°C.

11.10 Extraction of DNA from the clones of recombinant Lambda ZAP II

11.10.1 *Preparation of phage stock*

A well isolated single plaque was picked and placed into an Eppendorf tube containing 500 μl SM buffer. After incubation at 37° C for 1 hour, an aliquot of 25 μl of the phage stock was mixed with 100 μl of the freshly prepared suspension (OD_{600}=2.0) of XL1-Blue host bacteria and pre-incubated for 20 minutes at 37° C. The suspension was mixed with 3 ml of molten top agar and plated immediately onto a freshly poured 90 mm LB agar plate pre-warmed to 37°C. A total of 8 plates of each selected clone were prepared in this manner and incubated overnight at 37° C.

Next morning, the plates were removed from the incubator. Five ml of SM buffer was added to each plate and placed on a shaker at 30 rpm at 4oC for 4 hours. As much of the SM as possible was harvested from all the 8 plates with a pasteur pipette and pooled into a 50 ml polypropylene tube. One ml of SM was added to each plate again, rinsed around and the plate left in a tilted position for 15 minutes to allow all of the fluid to drain into one area. The SM was removed and pooled with the first harvest. Chloroform 0.8 μl was added to the pooled SM, mixed by brief vortexing and the tube centrifuged at 4,000 g for 10 minutes at 4oC. The supernatant (phage stock) was recovered, 8 drops of chloroform were added then the stock stored at 4oC.

11.10.2 *Preparation of phage DNA*

The phage stock was removed from the refrigerator and equilibrated to room temperature. Pancreatic DNAase I (104159, Boehringer) and RNAase (109126, Boehringer Mannheim), each was added to the phage stock to a final concentration of 1 μg/ml. After incubation for 30 minutes at room temperature, solid NaCl was added to a final concentration of 1 M, dissolved by swirling and then mixture left on ice for 1 hour. The solution was centrifuged at 11,000 g for 10 minutes at 4oC. The supernatant was replaced in a clean tube to which solid polyethylene glycol (P-5413, Sigma) was added to a final concentration of 10% (w/v). The polyethylene glycol was dissolved by rotating the tube on a spiramix (A 25 B, Denley, England). The solution was left on ice for 1 hour and then the precipitated phage particles were pelleted by centrifugation at 11,000 g for 10 minutes at 4oC. The supernatant was discarded and the tubes were placed in a tilted position for 5 minutes to allow the remaining fluid to drain away from the pellet. Using a wide-bore pipette equipped with a rubber bulb, the phage pellet was gently resuspended in 500 μl of SM buffer.

The polyethylene glycol and cell debris were removed from the phage suspension by chloroform extraction. An equal volume of chloroform was added to the suspension and vortexed for 30 seconds. The organic and aqueous phases were separated by centrifugation at 3,000 g for 15 minutes at 4oC. The extraction was repeated until there was no interface. The aqueous phase, which contained the phage particles was recovered.

To the suspension of purified phage particles, 20 μl of 10% SDS and 5 μl of 20 mg/ml proteinase K were added. After 1 hour of incubation at 37oC, the solution was extracted once with phenol, twice with a 50:50 mixture of phenol and chloroform and finally with chloroform alone. The DNA was precipitated by adding sodium acetate from a 3 M stock solution (pH 7.0) to a final concentration of 0.3 M and 2 volumes of ethanol. After leaving at room temperature for 30 minutes, the solution was centrifuged at 12,000 g for 2 minutes at 4oC. The pellet was washed with 70% ethanol and then redissolved in 2 ml of ultra pure water.

11.11 Evaluation of the Cloned DNA Probes

The probes prepared using the DNA of recombinant phages were subjected to hybridization to genomic DNAs from *Fasciola* spp. of different origin, and those from a number of host species, in a series of dilutions to evaluate their specificities and sensitivities.

11.11.1 *Preparation of slot-blots of genomic DNA*

A 2-fold serial dilution of the genomic DNA from 200 ng to 0.39 ng was prepared in 50 μl of ultra pure water. The DNA was sheared by syringe passing it through a fine needle (23 gauge) several times. The DNA was denatured by adding 20 μl of 0.3 N NaOH and heating. The solution was cooled quickly on ice and then 300 μl of neutralising buffer (2.5 ml 1 M Tris pH 7.4, 1.25 ml 2 M HCl, 6.5 ml x20 SSC) was added. The pH of the solution was checked by putting 10 μl on a pH indicator strip (31508, BDH). More buffer was added if the solution was not neutral.

One piece of nitrocellulose filter (S&S NC^{TM}, Schleicher & Schuell) and 2 pieces of blotter paper (GB002-SB, Schleicher & Schuell) were washed in x2 SSC. The nitrocellulose filter and blotter papers were placed in the blotter (Minifold II, Schleicher & Schuell). The apparatus was assembled according to the manufacturer's instructions and then attached to a water vacuum pump. First, 500 μl of x2 SSC was loaded to each sample slot and the water flow was adjusted to suck it in 5 minutes. The vacuum line was closed using a clamp and then the neutralised DNA samples were loaded to the sample slots. The clamp on the vacuum line was released. Once the sample was through, the nitrocellulose filter was removed from the apparatus, air dried, baked for 2 hours at 80oC, and then stored in an envelope at room temperature until processed for hybridization.

11.11.2 *Probing of genomic DNA with the recombinant phage DNA probes*

0.1 μg recombinant phage DNA was radiolabelled with [^{32}P] dCTP by nick translation as previously described (section 11.9.4). Hybridization between the radiolabelled probes and the various genomic DNAs immobilized on the slot-blots was allowed to proceed for at least 16 hours under the pre-hybridization and hybridization conditions described in section 11.9.5. The blots were washed, air dried and then autoradiographed as described earlier (section 11.9.6).

CHAPTER TWELVE

RESULTS

12.1 Storage of Fluke Samples

This experiment was designed to study the effects on the quantity and quality of the DNA extracted from the fluke specimens stored in 70% ethanol at 4°C for varying period of time. The amount of *F. gigantica* DNA obtained by phenol/chloroform extraction ranged from 2.280 to 3.196 mg per gram of frozen fluke material with an average of 2.910 mg (Table 12.1.1). The average yield of DNA was 0.304 mg per fluke (30-35 mm long *F. gigantica*) ranging from 0.253 to 0.342 mg per fluke.

Table 12.1.1. Yield of DNA from the *F. gigantica* specimens stored in 70% ethanol at 4°C for different periods.

Duration of storage	Weight of frozen flukes used for DNA extraction (g)*	Yield of DNA (mg)			Ratio of OD_{260} to OD_{280}
		Total	Per gram of fluke material	Per fluke	
0 month	0.555 (5)	1.710	3.110	0.342	1.90
1 month	0.641 (6)	2.040	3.180	0.340	2.06
2 months	1.000 (9)	2.280	2.280	0.253	1.81
3 months	0.665 (6)	1.660	2.496	0.277	1.78
4 months	0.488 (5)	1.560	3.196	0.312	1.93
5 months	0.572 (6)	1.820	3.182	0.300	1.78

* Figures in parenthesis indicate total number of frozen flukes used for DNA extraction.

There was no difference between the yield of DNA from the specimens which were freshly preserved in liquid nitrogen (0 month) and those which were stored in 70% ethanol at 4°C for 1 to 5 months (Table 12.1.1). Gel electrophoresis showed that all the DNA samples were of high molecular weight, undegraded and identical in quality (Figure 12.1.1).

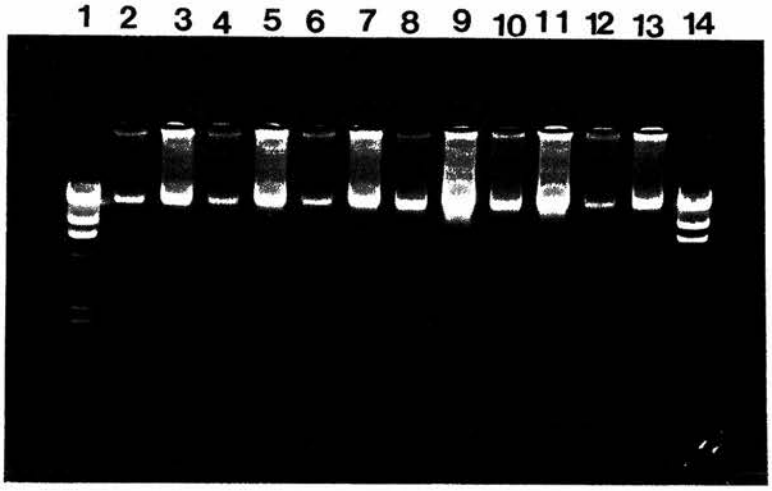


Figure 12.1.1. Agar gel electrophoresis of *F. gigantica* DNA isolated by phenol/chloroform extraction from the specimens stored in 70% ethanol at 4° C for 5 months (lanes 2-3 containing 0.1-1.8 μg DNA), 4 months (lanes 4-5 containing 0.5-1.6 μg DNA), 3 months (lanes 6-7 containing 0.5-1.7 μg DNA), 2 months (lanes 8-9 containing 0.5-2.3 μg DNA), 1 month (lanes 10-11 containing 0.5-2.0 μg DNA) and 0 month (lanes 12-13 containing 0.5-1.7 μg DNA); Lanes 1 and 14, each containing 0.5 λ DNA digested with Hind III as marker (fragment size from top to bottom: 23130, 9416, 6557, 4361, 2322 and 2027 base pairs).

12.2 Isolation of DNA

The yield of DNA isolated by different methods from parasite and host materials stored under different conditions are presented in 12.2.1.

Table 12.2.1. Details of the DNA samples used in the study.

Extraction Method	Material	Host	Origin	Storage condition	Quntity used for extraction	Yield of DNA (mg)	Ratio of OD260 to OD280
Equilibrium centrifugation in Cesium chloride	*F. hepatica*, fluke	Sheep	Scotland	Fresh	2 flukes	0.74	2.3
	F. gigantica, fluke	Cattle	Kenya	70% ethanol at room temp; 2 weeks	2 flukes	0.52	2.2
	F. gigantica, fluke	Buffalo	Nepal	70% ethanol at room temp; 4 weeks	1 fluke	0.33	1.8
	F. gigantica, fluke	Buffalo	Nepal	70% ethanol at room temp; 12 weeks	1 fluke	0.26	1.7
	F. gigantica, eggs	?	Malaysia	Dist. water in refrigerator; 4 years	Approx 0.5 ml	0.37	1.2
	F. gigantica, eggs	?	Zimbabwe	Dist. water in refrigerator; 4 years	Approx 0.5 ml	0.34	1.2
	Lymnaea spp, snail	NA	Nepal	70% ethanol at room temp; 2 weeks	Approx 1 ml tissue	0.32	1.4
	Buffalo liver	NA	Nepal	70% ethanol at room temp; 2 weeks	Approx 0.5 ml tissue	0.49	1.7
	Ovine liver	NA	Scotland	Fresh	Approx 0.5 ml tissue	0.96	1.8
	Bovine kidney	NA	Scotland	Fresh	Approx 1 ml tissue	2.34	2.7
Phenol/chloroform extraction	*F. gigantica*, fluke	Buffalo	Nepal	70% ethanol at room temp; 10 months	2 flukes	0.62	1.8
	F. gigantica, fluke	Sheep	Kenya	See Chapter 12 (Table 12.1.1)	37 flukes	11.07	1.9
	Caprine lymphocytes	NA	Scotland	Fresh	10 ml blood	0.28	1.9
	Bovine lymphocytes	NA	Scotland	Fresh	10 ml blood	0.20	1.6
CTAB precipitation	*F. hepatica*, fluke	Sheep	Scotland	Fresh	2 flukes	0.73	2.3
	F. gigantica, fluke	Cattle	Kenya	70% ethanol at room temp; 4 weeks	2 flukes	0.88	2.4

* Except for caprine and bovine blood, all the samples were kept later in liquid nitrogen until extraction of DNA; the storage conditions mentioned in this Table, therefore, refer to the conditions in which the samples were kept before the final storage; ? = not known; NA = not applicable.

Among the three methods used for extraction of DNA from fluke specimens, CTAB precipitation gave the highest yield (approximately 0.41 mg/fluke) followed by equilibrium centrifugation in $CsCl_2$ (approximately 0.31 mg/fluke) and phenol/chloroform extraction (approximately 0.30 mg/fluke). Also the OD_{260}/OD_{280} values of the DNA samples were higher when isolated by CTAB precipitation in comparison to those isolated by equilibrium centrifugation in $CsCl_2$ and phenol/chloroform extraction. Gel electrophoresis, showed that the DNAs isolated by all the three methods were undegraded and of high molecular weight.

12.3 Digestion of DNA with EcoR1

12.3.1 *Digestion under conventional conditions*

Gel electrophoresis demonstrated that *F. gigantica, F. hepatica* and bovine DNAs were digested under conventional conditions when EcoR1 was used at the concentrations of 2.5 and 5.0 units/µg of DNA in a 50 µl volume of reaction mixture and incubated at 37°C, either for 1 hour or 2 hours. In the second experiment, therefore, EcoR1 was used at a concentration of only 1 unit/µg of DNA and incubated at 37°C for varying periods of time between 1 and 22 hours. The DNAs cleaved well even at 1 hour of incubation.

12.3.2 *Digestion under star conditions*

In this experiment DNAs were digested with EcoR1 at an initial concentration of 15 units/µg of DNA for 4 hours. Then 1/3 volume of the digest was taken out and the remaining reaction mixture was recharged with the enzyme at a concentration 15 units/µg. Similarly, 1/2 of the volume of 6 hours digest was removed and the remaining reaction mixture was added with the enzyme at a concentration of 15 units/µg, and then the incubation was allowed to proceed for a further 16 hours. The DNAs were digested as early as 4 hours of incubation, however, addition of the enzyme and increase in the duration of incubation period resulted in smaller fragments.

12.4 Construction of DNA Libraries

12.4.1 *Size selection of Fasciola spp. DNA*

The yields of the EcoR1 digested fractionated DNA, by electro-elution from gels are shown in Table 12.4.1. The amounts of size selected DNA fragments were sufficient for ligation and preparation of DNA libraries.

Table 12.4.1. Recovery of size selected DNA by electro-elution from gels.

DNA spp.	Digestion condition	Size of the fragments (kb)	Quantity of digested DNA loaded on gel (μg)	Quantity of DNA recovered by electro-elution (μg)
F. hepatica	Conventional	0.5-1.0	24.0	4.2
		1.0-2.0	24.0	3.0
		2.0-4.0	24.0	3.6
	Star	0.5-1.0	14.0	2.2
		1.0-2.0	14.0	2.5
		2.0-4.0	14.0	1.9
F. gigantica	Conventional	0.5-1.0	24.0	3.6
		1.0-2.0	24.0	2.7
		2.0-4.0	24.0	2.4
	Star	0.5-1.0	14.0	1.7
		1.0-2.0	14.0	1.9
		2.0-4.0	14.0	1.7

12.4.2 *Titration of the DNA libraries*

Twelve libraries, six each of *F. hepatica* and *F. gigantica* DNAs were constructed in the vector λZap II using the 0.5-1.0, 1.0-2.0 and 2.0-4.0 kb fragments from each conventional and star digests. Titration showed that there was a big variation in phage titre of the libraries ranging from 5.0×10^4 to 3.5×10^6 pfu/μg of packaged DNA (Table 12.4.2). The ratios of recombinant to non-recombinant phages (white:blue plaques) varied from 0.3:1 to 88.0:1 and except for *F. gigantica* star digest libraries, these were relatively higher in those which consisted of bigger inserts (Table 12.4.2).

Table 12.4.2. Phage titre of the libraries and ratios of the recombinant to non-recombinant phages.

Library			Phage titre of the libraries			Recombinant to non-recombinant ratios
DNA spp.	Digestion condition	Size of insert (kb)	pfu/μl	pfu/library	pfu/μg of vector DNA	
F. hepatica	Conventional	0.5-1.0	1.4×10^2	7.0×10^4	1.8×10^5	0.3:1
		1.0-2.0	4.2×10^2	2.1×10^5	5.3×10^5	4.2:1
		2.0-4.0	7.7×10^2	3.9×10^5	9.8×10^5	18.0:1
	Star	0.5-1.0	2.0×10^2	1.0×10^5	2.5×10^5	0.4:1
		1.0-2.0	0.7×10^2	3.5×10^4	8.8×10^4	2.2:1
		2.0-4.0	9.4×10^2	4.7×10^5	1.2×10^6	20.3:1
F. gigantica	Conventional	0.5-1.0	1.4×10^2	7.0×10^4	1.8×10^5	1.4:1
		1.0-2.0	1.8×10^2	9.0×10^4	2.3×10^5	5.0:1
		2.0-4.0	2.7×10^3	1.4×10^6	3.5×10^6	88.0:1
	Star	0.5-1.0	3.6×10^2	1.8×10^5	4.4×10^5	2.3:1
		1.0-2.0	6.4×10^2	3.2×10^5	8.0×10^5	2.0:1
		2.0-4.0	0.4×10^2	2.0×10^4	5.0×10^4	0.7:1

12.5 Screening of Libraries

In all, 6091 plaques from 35 plates were screened with ^{32}P-labelled *F. gigantica*, *F. hepatica*, bovine, caprine and ovine DNAs of which 1190 reacted with *Fasciola* spp. DNA (Table 12.5.1). Four of these hybridising plaques were selected for further study. The first of these, designated MHFh (from 2.0-4.0 kb star digest library of *F. hepatica*) was strongly reactive with *F. hepatica* DNA only, while the second, designated MHFg (from 2.0-4.0 kb conventional digest library of *F. gigantica*) reacted with *F. gigantica* DNA only. The third, designated as MHFx1 (from 1.0-2.0 kb star digest library of *F. hepatica* and fourth, designated as MHFx2 (from 1.0-2.0 kb conventional digest library of *F. gigantica*) were strongly reactive with the both *F. hepatica* and *F. gigantica* DNA. None of these plaques reacted with bovine, caprine and ovine DNAs tested.

Table 12.5.1. Results of primary screenings of the libraries.

Library			Number of plaques Screened	Number of hybridising plaques				
DNA spp.	Digestion condition	Size of insert (kb)		Specific		Cross-reactive		Total
				Strong	Weak	Strong	Weak	
F. hepatica	Conventional	0.5-1.0	193	0	0	0	4	4
		1.0-2.0	159	0	2	1	2	5
		2.0-4.0	294	1	0	4	21	26
	Star	0.5-1.0	183	0	0	0	2	2
		1.0-2.0	162	1	1	1	4	7
		2.0-4.0	473	1	1	4	36	42
F. gigantica	Conventional	0.5-1.0	191	0	1	0	3	4
		1.0-2.0	999	0	9	14	168	191
		2.0-4.0	2142	4	29	94	523	650
	Star	0.5-1.0	154	0	3	0	0	3
		1.0-2.0	964	1	9	21	215	246
		2.0-4.0	177	0	1	1	8	10
		TOTAL	6091	8	56	140	986	1190

12.6 Isolation of MHFh, MHFg, MHFx1 and MHFx2 DNA Inserts

An attempt was made to isolate DNA inserts from the MHFh, MHFg, MHFx1 and MHFx2 clones. DNAs from the clones were digested with EcoR1 under conventional and star conditions and then fractionated by 0.8% agarose gel electrophoresis. Although *F. gigantica* DNA insert in the MHFg clone was found to have a molecular weight of approximately 3 kb, the result was not conclusive for other clones, possibly due to improper digestion of the DNAs or contamination of the samples. Because of time constraints, however, the experiment could not be repeated.

12.6 Specificity and Sensitivity of MHFh, MHFg, MHFx1 and MHFx2 Probes

In order to further study the specificity and sensitivity, slot-blot analysis of doubling dilutions (200.0-0.4 ng) of *F. hepatica* DNA isolated from adult flukes (Scotland), *F. gigantica* DNA isolated from adult flukes (Kenya and Nepal) and eggs (Malaysia and Zimbabwe), and hosts (cattle, buffalo, goat, sheep and *Lymnaea* spp.), DNAs were carried out using [^{32}P]-labelled bacteriophage DNA containing the cloned inserts as probes. Under the conditions specified, the MHFh probe hybridized solely with DNA from *F. hepatica* and detected as little as 3.0 ng DNA (Figure 12.5.1). While the second probe, MHFg reacted strongly with *F. gigantica* DNA up to the dilution of 12.0 ng, it also reacted very weakly with *F. hepatica* DNA up to the dilution of 100.0 ng. The cross-reactive probes, MHFx1 and MHFx2 easily detected 25.0 ng DNA of both the *Fasciola* spp. The various isolates of *Fasciola* spp. DNA tested yielded similar results with the four probes. None of the host DNAs tested reacted with probes on hybridization, the background for these was similar to that in Figure 12.5.1, Track 2.

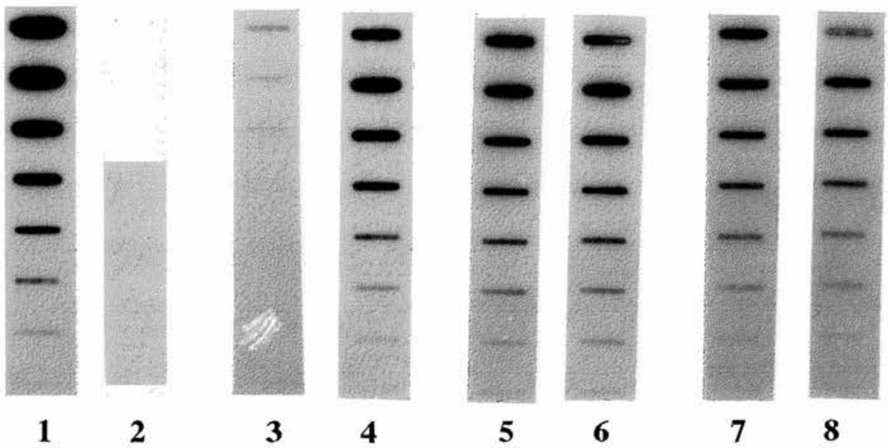


Figure 12.5.1. Specificity and sensitivity of the MHFh, MHFg, MHFx1 and MHFx2 Probes. Doubling dilutions (200.0-1.6 ng) of *F. hepatica* (Tracks 1, 3, 5 and 7) and *F. gigantica* (Tracks 2, 4, 6 and 8) DNA blotted onto nitrocellulose then probed with [^{32}P]-labelled bacteriophage DNA containing the *F. hepatica* specific insert, MHFh (200 ng at 3.3 x 10^7 dpm/μg exposed for 72 hours and developed for 2 min- Tracks 1 and 2), the *F. gigantica* specific insert, MHFg (200 ng at 3.1 x 10^7 dpm/μg exposed for 72 hours and developed for 2 min- Tracks 3 and 4), and the cross-reactive inserts, MHFx1 (200 ng at 1.8 x 10^7 dpm/μg exposed for 72 hours and developed for 2 min-Tracks 5 and 6) and MHFx2 (200 ng at 1.2 x 10^7 dpm/μg exposed for 72 hours and developed for 2 min-Tracks 7 and 8). Hybridization with host i.e. bovine, ovine, caprine and *Lymnaea* spp. DNA were negative as in Track 2.

CHAPTER THIRTEEN

DISCUSSION

In epidemiological studies, it is imperative to know accurately the identity of the causative organisms and the extent of their geographic distribution. Where species can be identified by morphological means, the labour and expense of nucleic acid based methods is neither necessary nor justifiable. However, there are circumstances where morphology cannot be used or is unreliable. For example, a range of morphological types of *Fasciola* spp. occurs in South and Southeast Asia (Chapter 10). A similar situation was observed during the course of the present epidemiological study (Chapter 4). Various alternative methods, such as cytology and biochemistry have failed to resolve the controversial status of the common liver flukes in Japan. In such situations, DNA probes offer a real solution.

Among the four DNA probes developed in this study, two are species-specific and two are cross-reactive. While the MHFh probe is strictly specific to *F. hepatica*, the MHFg can positively identify *F. gigantica* provided the test blot contains less than 100 ng DNA. Thus, if used in conjunction, these two probes can unequivocally differentiate between *F. hepatica* and *F. gigantica* by slot-blot or dot-blot analysis. Both of these probes are highly sensitive and can clearly detect 25 ng DNA of the respective species of *Fasciola*.

Kendall (1965), Boray (1966) and Malek (1980) suggested that intraspecific diversities of *Fasciola* spp. may have been induced by geographical isolation and adaptation to different definitive hosts, *Lymnaea* spp. and to their environments. Views of these workers have been supported by Alcaino, Baker and Fisk (1976) and Agatsuma and Suzuki (1980) who found variations in isoenzyme patterns reflecting the geographical origin of the hosts. It is possible, therefore, that the invasiveness and pathogenicity of *Fasciola* spp. may also vary within its very wide geographical range. However, the intraspecific variations of *Fasciola* spp. at the molecular level have been poorly studied, largely because homologous DNA probes are not available (Blair and

McManus, 1989). Cross-reactive DNA probes developed in this study such as MHFx1 and MHFx2 could make a major impact in epidemiological studies as reagents for the identification of discrete strain or geographical isolates of *Fasciola* spp. through restriction fragments length polymorphism. The probes hybridized with isolates from Kenya, Zimbabwe, Malaysia, Nepal and Scotland which indicates that these could be used in world-wide epidemiological studies.

Finding naturally infected snails in the field in connection with vector identification, or seasonal transmission studies, is difficult. The usual procedures involve examination of living specimens to determine if they are shedding cercariae and subsequent experimental infection of definitive hosts with the metacercariae. Detection of early intramolluscan infections of the parasite is difficult because it requires examination of serial sections of fixed and histologically stained tissue of snails. Crucially, these methods are not specific and cannot be used to identify the species of *Fasciola* infecting the snails. Available information suggests that there are no profound DNA rearrangements between the various life cycle stages of parasites (Harrison, 1991). So that the same DNA is present whether the parasite is in its definitive, intermediate or vector hosts or indeed in its free-living stages. It appears, therefore, that the DNA probes prepared in this study could eliminate both the inherent uncertainties of visual examination and the extensive incubation period required in the current tests for the presence of *Fasciola* spp. and their identification in the snail vector. An oligonucleotide probe developed by Shubkin et al. (1992) can detect *F. hepatica* in its intermediate host, however, this may not be suitable to be used in the areas where *F. gigantica* is a sole or predominant species.

The present study showed that fluke specimens can be preserved in 70% ethanol at 4°C for at least 5 months without deterioration in quantity and quality of DNA. Indeed, the DNA extracted from the fluke specimens which were kept at room temperature for 10 months in Nepal had no qualititative or quantitative deteriorations. Similarly, the *Fasciola* eggs which were stored in distilled water at 4°C for approximately 4 years yielded good quality DNA. The DNA obtained from one fluke (30-35 mm long) or about 0.5 ml of the eggs (sedimented) was nearly 12000-14000 times more than the amount required to identify *Fasciola* spp. by slot-blot or dot-blot.

It appears, therefore, that the reagents developed in this study now allow greater flexibility and ease in transporting samples from post mortem rooms, slaughter places, abattoirs or the field when compared with the often impractical procedures of transportation at 4°C or subzero temperatures. It will facilitate the collection and identification of substantially more isolates than hitherto examined. With DNA probes such as those described in this work, new and valuable research into the epidemiology of *Fasciola* spp., vector suitability, and the host-parasite relationship will become possible.

GENERAL DISCUSSION

CHAPTER FOURTEEN

GENERAL DISCUSSION

The results of this study confirm that fasciolosis is endemic in eastern Nepal. However, the increased awareness of the disease and massive increase in the use of anthelmintics appear to have made no impact on the prevalence since 1973 (Singh et al., 1973). The similarities in the prevalences recorded in this study and those reported from the other regions of Nepal indicate that the picture which emerged from the present survey possibly represents the overall national trend.

There was a widespread opinion, but little supportive data that fasciolosis is an economically important helminth infection in ruminants in Nepal. There were no records of animals dying of fasciolosis in spite of the high prevalence. The results of the present experimental infections indicated that many buffaloes, cattle, goats and sheep may die from fasciolosis. In eastern Nepal, where average *F. gigantica* burdens may exceed 200 (1-2071) flukes in buffaloes and 23 (1-179) flukes in goats which are invariably kept under poor nutritional conditions, a significant mortality may not be uncommon. Quantification of such losses, however, is impossible because data on mortalities are not available due to poorly developed veterinary services and no disease-reporting procedures. As it is unlikely that the situation will improve in the near future, estimates of the mortality in buffaloes and goats could be made by a pilot questionnaire survey because these animals, if in *extremis* due to any disease are generally slaughtered on-farm.

The results of the experimental studies clearly demonstrated that liveweight gain of an infected animal is reduced even in sub-clinical infections. As no figures are available to allow estimation of the crude economic significance of fasciolosis in Nepal, an alternative method would be to extrapolate data on reduced liveweight from the experimental studies to the natural situation.

Regression analysis indicated that in buffalo infected with *F. gigantica* each fluke reduced the annual liveweight gain by 338 g which is equivalent to about 152 g meat (killing out 45% of liveweight). In Nepal, 760000 buffaloes are slaughtered annually which produce about 95000 tonnes of meat (FAO, 1991). Assuming an 80% infection rate and a mean burden of 200 flukes which were observed in the present epidemiological study, there would be a loss of about 18483 tonnes of buffalo meat or some NRs.646905000 (at NRs.35/kg meat) or US$14,375,667 (at the current exchange rate of US$1 = NRs.45).

In goats, the regression indicated that each *F. gigantica* reduced the annual liveweight gain by 312 g or 156 g meat (killing out 50% of liveweight). Thus, the loss caused by fasciolosis in 3,220,000 goats slaughtered annually (FAO, 1991) with a prevalence of 35% and a mean burden of 24 flukes (Chapter 4, Section 4.5), would be 4220 tonnes of goat meat or NRs.274300000 (at NRs 65/kg meat) or US$6,095,556.

It is not suggested that the estimates of financial loss resulting from reduced liveweight gain in buffaloes and goats, currently valued at NRs.921205000 (US$20,471,223) is an accurate assessment because the calculations are inevitably based on several major assumptions, such as the prevalence and the mean fluke burdens in eastern Nepal are representative of the whole country, nutritional status of the experimental buffaloes and goats were representative for the whole country and all the slaughtered animals had the same fluke burden for the whole year. Some of these assumptions may not be true, and in particular, it must be pointed out that the experimental animals were kept on a high plane of nutrition which is very rare in the on-farm conditions. However, this estimate gives some indication of the enormity of the problem in Nepal when one compare this with the per capita GNP (gross national product) of only US$180 in 1991 (CBS, 1993).

A review of the literature indicated that fasciolosis has profound impacts on milk and wool production and reproductive efficiency; the studies are mainly all based on fasciolosis caused by *F. hepatica*. However, published literature and the present study clearly indicate that *F. gigantica* is more pathogenic than *F. hepatica* suggesting that the extrapolation of published data to estimate the financial loss caused by *F. gigantica* infections would be an underestimate. Furthermore, it appears that no studies have been carried out to assess the effects of fasciolosis on working efficiency of draught animals. A decreased level of haemoglobin concentration in the infected animal will affect the carrying capacity of oxygen and may lead to reduced work output. Therefore, to elucidate the impact of fasciolosis on the Nepalese economy, further studies on these aspects are necessary.

Besides the species and age of the definitive hosts, the density of *Lymnaea* habitats and source of food/grazing site were the major determinants of prevalence, these in turn, were influenced by the season and the farming systems. Below 1800 m, water temperatures found in natural habitats appeared to have little direct effect on *Lymnaea* spp. However, it is likely, that the development of the larval stages of *Fasciola* spp. in the intermediate hosts is arrested or delayed during the cold winter season.

From the epidemiological point of view, the pre-monsoon and the monsoon rains, together with rice cultivation practices were important factors which create numerous temporary *Lymnaea* habitats over a wide area and disperse the infected snails to these habitats. Both these factors prevented grazing of animals near the habitats during the mid and late monsoon, thus preventing contamination with *Fasciola* eggs and also animal infection. However, after the monsoon when rice is harvested, the road-sides and rice-fields which are heavily contaminated with metacercariae are available for grazing. Keeping the animals away from these areas during the pre-monsoon and again after the monsoon until the end of the viable period of the metacercariae would be of great value in the control of fasciolosis. However, this might not be acceptable to farmers as alternative forage resources are scarce during these periods.

Rice-straw is another important source of infection. The prolonged survival of *F. gigantica* metacercariae encysted on the rice-straw kept in barns where the relative humidity was more than 60% has been reported by several workers (Kimura and Shimizu, 1978; Abu and Shiramizu, 1985; Shiramizu and Abu, 1988). In eastern Nepal, where the relative humidity is generally above 60% during the winter, the metacercariae may remain infective for a considerable period of time. However, further research is needed to determine the viability of the metacercariae in different climatic conditions so that recommendation for a safe period for rice-straw feeding could be made. Alternatively, farmers could be advised to cut the straw into two halves at the time of harvest. The top half which is expected to be free from the infective metacercariae could then be used for feeding animals during the winter season while the bottom half which is generally infected should be used later during the summer and the monsoon after the metacercariae have died.

Attempting to control snails using molluscicides during the dry season would be of doubtful value due to the large number of permanent habitats, the great biotic potential of the snails and recurrent labour and equipment costs. Furthermore, chemical molluscicides may be toxic for mammals and their drainage into streams may kill fish and other aquatic fauna including frogs and insects.

Planting certain tree spp., the leaves and seeds of which are molluscicidal such as *Eucalyptus* spp. (J.A. Hammond, 1993, personal communication), *Sapindus emarginatus, Acacia concinna, Caesalpinia corearia* and *Embelica officinalis* (Bali et al., 1985) round the edges of habitats has been found as a potential method of vector snail control. It seems unlikely that farmers in the hills would plant trees around springs in their rice-fields because of the adverse effects of shade on rice production. However, tree species with marked molluscicidal properties could be included in the present on-going road-sides and canal bank plantation programme run by the Ministry of Forest and Environment in the Terai.

As previously reported (Morel and Mahato, 1987), the present study also suggests that the bulk of the infection in snails is derived from fluke eggs deposited on the pasture during March-May and again in October and November. Thus there is a good case for administration of anthelmintics in February and in late August to control the pasture contamination. However, this approach would only be effective if all stock including goats and sheep were treated with an efficient anthelmintic. The potential of this strategic dosing regime using locally available anthelmintics, including its cost-effectiveness for long term control of fasciolosis, however, must be studied in pilot experiments in the different agro-ecological conditions before being recommended for large scale application.

Predictive models which forecast the incidence of *F. hepatica* fasciolosis for the impending year have been developed and used in temperate climates for some time. The use of such models allows the risk of a serious outbreak of disease to be assessed quantitatively, so that strategic dosing regimes and other suitable prophylactic actions for the forthcoming year can be selected. To date, there has been little attempt to adapt *F. hepatica* fasciolosis forecast models for use in tropical climates. It is not known whether the existing climate forecast models which are currently in use in temperate climates, can be directly applied to Nepal. An expert system which is based on the associations between meteorological, geographical, topographical, snail habitat extent, farming systems etc. data, and patterns in the disease could possibly be developed using geographical information systems. In an expert system, stocking rate, the effect of fluke burdens on productivity, cost of control methods and economic return could easily be incorporated (Meek and Morris, 1981). The system could then be used to advise farmers on the most appropriate control strategy, which could have a profound impact on fasciolosis and the associated losses in animal production, as well as improving the cost-effectiveness of the control measures undertaken.

REFERENCES

REFERENCES

Abu, M. and Shimizu, K. (1985) Preventive investigation of bovine fascioliasis I. Survey of temperature and moisture of straw bundles in mountainous area of Yamaguchi prefecture. *Yamaguchi Journal of Veterinary Medicine*, **12**: 89-96.

Agatsuma, T. (1981) Electrophoretic studies on enzymes in the Japanese common liver fluke, *Fasciola* sp. II. On further three enzymes, GPI, GDH and ME, in the liver fluke. *Japanese Journal of Parasitology*, **30**: 157-159.

Agatsuma, T. and Suzuki, N. (1980) Electrophoretic studies on enzymes in the Japanese common liver fluke, *Fasciola* sp. I. Enzyme variation in the natural population. *Japanese Journal of Medical Science and Biology*, **33**: 249-254.

Agrawal, M.C., Bhandari, K.S. and Chaudhry, R.K. (1987) Preliminary studies on molluscicidal properties of seed-cake of Mahua (*Madhuca indica*). *Indian Journal of Animal Science*, **57**: 546-547.

Ajanusi, O.J., Ogunsusi, R.A., Njoku, C.O. and Gyang, E.O. (1988). *Fasciola gigantica*: Pathological and helminthological observations in experimental infection of Yankassa lambs. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux*, **41**: 381-386.

Akahane, H. (1975) The characteristic changes of haematological findings and immunological response of the rabbits infected with *Fasciola* sp. by migration of flukes in the liver. 1. The characteristic changes of haematological findings. *Japanese Journal of Parasitology*, **24**: 347-352.

Akahane, H. and Oshima, T. (1976) Patterns of the variation of the common liver fluke (*Fasciola* spp.) in Japan. *Japanese Journal of Parasitology*, **25**: 231-234.

Alcaino, H.A., Baker, N.F. and Fisk, R.A. (1976) Enzyme polymorphism in *Fasciola hepatica* L.: Esterases. *American Journal of Veterinary Research*, **37**: 1153-1157.

Alexander, H.D. and Kiesel, G.K. (1965) The effect of blood loss on weight gain, haemoglobin and haematocrit in lambs fed different levels of protein. *Auburn Veterinarian*, **12**: 114-129.

Alicata, J.E. (1938) Observations on the life history of *Fasciola gigantica*, the common liver fluke of cattle in Hawaii, and the intermediate host, *Fossaria ollula*. *Bulletin of Hawaii Agricultural Experiment Station*, No. 80.

American Association of Veterinary Parasitologists (1983) Research need and priorities for ruminant internal parasites in the United States. *American Journal of Veterinary Research*, **44**: 1836-1847.

Anderson, P.H., Berrett, S. and Patterson, D.S.P. (1978) Resistance to *Fasciola hepatica* in cattle. *Journal of Comparative Pathology*, **88**: 245-251.

Anon (1979) Livestock Development Project Appraisal. Department of Livestock Development and Animal Health, Ministry of Agriculture, His Majesty's Government of Nepal.

Anon (1984) Annual Report 1983-84. Animal Disease Investigation and Parasite Control Division, Kathmandu, Nepal.

Archer, R.K. (1963) *The Eosinophil Leucocytes*. Blackwell Scientific Publications, Oxford.

Armour, J. and Dargie, J.D. (1974) Immunity to *Fasciola hepatica* in rat: successful transfer of immunity by lymphoid cells and serum. *Experimental Parasitology*, **35**: 381-388.

Armour, J., Dargie, J.D., Doyle, J.J., Murray, M., Robinson, P. and Rushton, B. (1974) Immunisation against fascioliasis. *Proceedings of the Third International Congress of Parasitology*, Munich, Vol. 1,p. 494.

Babalola, D.A. and Schillhorn van Veen, T.W. (1976) Incidence of fasciolosis in cattle slaughtered in Bauchi (Nigeria). *Tropical Animal Health and Production*, **8**: 243-247.

Bagadi, H.O. (1970) The pathogenesis of infectious necrotic hepatitis, black disease, of sheep. Ph.D. Thesis. University of Edinburgh.

Bagadi, H.O. (1974) Infectious necrotic hepatitis (black disease) of sheep. *Veterinary Bulletin*, **44**: 385-388.

Bagadi, H.O. and Sewell, M.M.H. (1973) Experimental studies on infectious necrotic hepatitis (black disease) of sheep. *Research in Veterinary Science*, **15**: 53-61.

Baker, D.C. (1987) Molecular approaches to DNA Diagnosis. *Parasitology*, **99**: S125-S146.

Bali, H.S., Singh, S. and Pati, S.C. (1985) Preliminary screening of some plants for molluscicidal activity against two snail species. *Indian Journal of Animal Science*, 55(5): 338-340.

Basten, A. and Beeson, P.B. (1970) Mechanism of eosinophilia. II. Role of the lymphocytes. *Journal of Experimental Medicine*, **131**: 1288-1305.

Basu, A.K. and Bandyopadhyay, P.K. (1986) Jaundice in cattle due to *Fasciola* infection. *Current Science, India*, **55**: 578-579.

Behm, C.A. and Bryant (1982) Phosphoenolpyruvate carboxykinase from *Fasciola hepatica*. *International Journal of Parasitology*, **12**: 271-278.

Berry, C.I. and Dargie, J.D. (1976) The role of host nutrition in the pathogenesis of ovine fasciolosis. *Veterinary Parasitology*, **2**: 317-332.

Besedovsky, H., Del Rey, A., Sorkin, E. and Dinarello, C.A. (1986) Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science*, **233**: 652-654.

Bhattacharjee, M.L. and Halder, B.R. (1971) The occurrence of *Fasciola gigantica* in the liver of an Indian rhinoceros (*R. unicornis*). *British Veterinary Journal*, **127**: vii-viii.

Bindernagel, J.A. (1972) Liver fluke *Fasciola gigantica* in African buffalo and antelopes in Uganda, East Africa. *Journal of Wildlife Diseases*, **8**: 315-317.

Bitakaramire, P.K. (1969) Studies on *Fasciola gigantica* infection of cattle. Ph.D. Thesis. University of East Africa.

Bitakaramire, P.K. (1973a) The incidence of fascioliasis in different breeds of cattle in Kenya. *Bulletin of Epizootic Diseases of Africa*, **15**: 389-391.

Bitakaramire, P.K. (1973b) Preliminary studies on immunization of cattle against fasciolosis using gamma-irradiated metacercariae of *Fasciola gigantica*. In: *Isotope and Radiation in Parasitology*, iii: 23-32.

Black, N.M. and Froyd, G. (1972) The possible influence of liver fluke infestation on milk quality. *Veterinary Record*, **90**: 71-72.

Blair, D. (1993) Molecular variation in fasciolid and *Paragonimus*. *Acta Tropica*, **53**: 277-289.

Blair, D. and McManus, D.P. (1989) Restriction enzyme mapping of ribosomal DNA can distinguish between fasciolid (liver fluke) species. *Molecular and Biochemical Parasitology*, **36**: 201-208.

Blamire, R.V., Goodhand, R.H. and Tailor, K.C. (1980) A review of some animal diseases encountered in meat inspections in England and Wales, 1969-1978. *Veterinary Record*, **106**: 195-199.

Bohlender, R.E. (1988) Economics of deworming beef cattle. *Veterinary Parasitology*, **27**: 67-71.

Blood, D.C. and Radostitis, O.M. (1989) Diseases caused by *Clostridium* spp. In: *Veterinary Medicine*, 7th edn, pp. 597-612. Bailliere Tindall, London.

Boag, D.A. (1986) Dispersal in pond snails: potential role of waterfowl. *Canadian Journal of Zoology*, **64**: 904-909.

Bolbol, A.S. (1975) The immunology of fascioliasis in rabbits. Ph.D. Thesis, University of Edinburgh.

Boray, J.C. (1963) The ecology of *Fasciola hepatica* with particular reference to its intermediate host in Australia. *Proceedings of 17th World Veterinary Congress*, Hanover, Vol. 1, pp. 709-715.

Boray, J.C. (1966) Studies on the relative susceptibility of some lymnaeids to infection with *Fasciola hepatica* and *F. gigantica* and on the adaptation of *Fasciola* spp. *Annals of Tropical Medicine and Parasitology*, **60**: 114-125.

Boray, J.C. (1967) Studies on experimental infections with *Fasciola hepatica*, with particular reference to acute fascioliasis in sheep. *Annals of Tropical Medicine and Parasitology*, **61**: 439-450.

Boray, J.C. (1969) Experimental fascioliasis in Australia. *Advances in Parasitology*, **7**: 95-210.

Boray, J.C. (1982) Fascioliasis. In: *Handbook Series in Zoonoses*, (eds. G.V. Hillyer and C.E. Hopla), Vol. III, pp. 71-88.. CRC Press Inc, Florida, USA.

Boray, J.C. (1985) Flukes of domestic animals. In: *Parasites, Pests and Predators*, (eds. S.M. Gaafar, W.E. Howard and R.E. Marsh), World Animal Science, B2. pp. 179-218. Elsevier Science Publishers, Amsterdam.

Boray, J.C. and Enigk, K. (1964) Laboratory studies on the survival and infectivity of *Fasciola hepatica* and *F. gigantica* metacercariae. *Zeitschrift fur Tropenmedezin und Parasitologie*, **15**: 324-331

Boycott, A.E. (1936) The habitats of freshwater Mollusca in Britain. *Journal of Animal Ecology*, **5**: 116-186.

Brown, D.S. (1980) Chemical and physical factors. In: *Freshwater Snails of Africa*, pp. 345-368. Taylor & Francis, London.

Bryant, C. and Williams, J.P.G. (1962) Some aspects of the metabolism of the liver fluke, *Fasciola hepatica*. *Experimental Parasitology*, 12: 372-376.

Bryceson, A.D., Warrell, D.A. and Pope, H.M. (1977) Dangerous reactions to treatment of onchocerciasis with carbamazine. *British Medical Journal*, **i**: 742-744.

Burden, D.J. and Hammet, N.C. (1978) Microplate enzyme linked immunosorbent assay for antibody to *Fasciola hepatica* in cattle. *Veterinary Record*, **103**: 158.

Burden, D.J., Hughes, D.L., Hammet, N.C. and Collis, K.A. (1978) Concurrent daily infection of calves with *Fasciola hepatica* and *Ostertagia ostertagi*. *Research in Veterinary Science*, **25**: 302-306.

Burden, D.J., Bland, A.P., Hammet, N.C. and Hughes, D.L. (1983) *Fasciola hepatica*: migration of newly excysted juveniles in resistant rats. *Experimental Parasitology*, **56**: 277-288.

Butterworth, A.E. (1984) Cell-mediated damage to helminths. *Advances in Parasitology*, **23**: 143-235.

Campbell, N.J., Kelly, J.D., Townsend, R.B. and Dineen, J.K. (1977) The stimulation of resistance in sheep to *Fasciola hepatica* by infection with *Cysticercus tenuicollis*. *International Journal of Parasitology*, **7**: 347-351.

Cannon, R.M. and Roe, R.T. (1982) *Livestock Disease Survey: A Field Manual for Veterinarians*. Australian Government Publishing Service, Canberra.

Castelino, J.B. and Preston, J.M. (1979) The influence of breed and age on the prevalence of bovine fascioliasis in Kenya. *British Veterinary Journal*, **135**: 198-203.

CBS (1993) *Statistical Year Book of Nepal 1993*. Central Bureau of Statistics, National Planning Commission Secretariat, His Majesty's Government of Nepal.

Chaudhri, S.S., Mandokhot, V.M., Gupta, R.P and Yadav, C.L. (1988) Haematological and biochemical observation in buffaloes naturally infected with *Fasciola gigantica*. *Indian Veterinary Journal*, **65**: 23-27.

Chaudhri, S.S., Gupta, R.P., Kumar, S., Singh, J. and Sangwan, A.K. (1993) Epidemiology and control of *Fasciola gigantica* infection of cattle and buffaloes in eastern Haryana, India. *Indian Journal of Animal Sciences*, **63**: 600-605.

Chernin, E. and Dunavan, C.A. (1962) The influence of host-parasite dispersion upon the capacity of *Schistosoma mansoni* miracidia to infect *Australorbis glabratus*. *American Journal of Tropical Medicine and Hygiene*, **11**: 455-471.

Cheruiyot, H.K. and Wamae, L.W. (1989) Incidence of *Chaetogaster limnaei* (Annelida: Oligochaeta) in common fresh water snails in Karatina, Kenya. *Bulletin of Animal Health and Production in Africa*, **37**: 175-177.

Chick, B.F., Coverdale, D.R. and Jackson, A.R.B. (1980) Production effects of liver fluke (*Fasciola hepatica*) infection in beef cattle. *Australian Veterinary Journal*, **56**: 588-592.

Christensen, N.O., Nansen, P. and Frandsen, F. (1976b) The influence of temperature on the infectivity of *Fasciola hepatica* to *Lymnaea truncatula*. *Journal of Parasitology*, **62**: 698-701.

Christensen, N.O., Nansen, P. and Frandsen, F. (1976a) Molluscs interfering with the capacity of *Fasciola hepatica* miracidia to infect *Lymnaea truncatula*. *Parasitology*, **73**: 161-167.

Christensen, N.O., Nansen, P. and Frandsen, F. (1978) The influence of some physico-chemical factors on host-finding capacity of *Fasciola hepatica* miracidia. *Journal of Helminthology*, **52**: 61-67.

Chubaryan, F.A. (1964) [Digestion and nitrogen balance in fascioliasis of sheep]. *Izvestiia Akademii Nauk aryan, SSR*, **17**: 51-58. [In Russian]

Colley, D.G. (1972) *Schistosoma mansoni*: eosinophilia and the development of lymphocyte blastogenesis in response to soluble egg antigen in inbred mice. *Experimental Parasitology*, **32**: 520-526.

Colley, D.G. (1974) Variations in peripheral blood eosinophil levels in normal and *Schistosoma mansoni*-infected mice. *Journal of Laboratory and Clinical Medicine*, **83**: 871-876.

Corba, J., Armour, J., Roberts, R.J. and Uquhart, G.M. (1971) Transfer of immunity to *Fasciola hepatica* infection by lymphoid cells. *Research in Veterinary Science*, **12**: 292-295.

Coulter Electronics (1985) *Reference Manual for the Coulter Counter Model ZM*. Coulter Electronics Ltd., Luton, England.

Coyle, T.J. (1958) Experiments in the diagnosis and treatment of fascioliasis in Uganda cattle. *Bulletin of Epizootic Diseases of Africa*, **6**: 255-272.

Coyle, T.J. (1959) Control of fascioliasis in Uganda. CCTA Publication No. 49. IACED Symposium on Helminthiasis, Nairobi, 1959.

Coyle, T.J. (1961) The epidemiology of Fasciola gigantica in cattle in Uganda Protectorate. Thesis. Royal College of Veterinary Surgeons.

Cridland, C.C. (1967) Resistance of *Bulinus (P) globosus, B. (P) africanus, Biomphalaria pfeifferi* and *Lymnaea natalensis* to experimental desiccation. *Bulletin of World Health Organization*, **36**: 507-513.

Crossland, N.O., Johnstone, A., Beaumont, G. and Bennett, M.S. (1977) The effect of control of chronic fascioliasis on the productivity of lowland sheep. *British Veterinary Journal*, **133**: 518-525.

Dacie, J.V. and Lewis, S.M. (1963) *Practical Haematology*, 3rd edn, pp. 58-60. J. & A. Churchill Ltd. London.

Dargie, J.D., Berry, C.I. and Parkins J.J. (1979) The patho-physiology of ovine fascioliasis: studies on the feed intake and digestibility, body weight and nitrogen balance of sheep given rations of hay or hay plus a pelleted supplement. *Research in Veterinary Science*, **76**: 289-295.

Dargie, J.D. (1987) The impact on production and mechanisms of pathogenesis of trematode infections in cattle and sheep. *International Journal of Parasitology*, **17**: 453-463.

Dargie, J.D., Holmes, P.H., MacLean, J.M. and Mulligan, W. (1968) Further studies of anaemia in fascioliasis: simultaneous use of ^{51}Cr labelled red cells and ^{95}NB labelled albumin. *Veterinary Record*, **LXXXII**: 360-361.

Dargie, J.D. and Mulligan, W. (1971) Ferrokinetic studies on normal and fluke-infected rabbits. *Journal of Comparative Pathology*, **80**: 37-45.

Dargie, J.D., Armour, J. and Uquhart, G.M. (1973) Studies on immunity to *Fasciola hepatica*. *Parasitology*, **67**: 25-32.

David, J.R., Butterworth, A.E. and Vadas, M.A. (1980) Mechanism of the interaction mediating killing of *Schistosoma mansoni* by human eosinophils. *American Journal of Tropical Medicine and Hygiene*, **29**: 842-848.

Davies, C. and Goose, J. (1981) Killing of newly excysted juveniles of *Fasciola hepatica* in sensitised rats. *Parasite Immunology*, **3**: 81-86.

Davtyan, E.A. (1953) Acute form of fascioliasis in sheep. Paper presented to Academician K.I. Skryabin on his 75th birthday. Izdatelstvo Akademii Nauk SSSR, Moscow. [In Russian].

Davtyan, E.A. (1956) Pathogenicity of different species of *Fasciola* and its variability depending on the developmental conditions of yhe parthenogenetic stages. *Zoologicheskii Zhurnal*, **35**: 1617-1625. [In Russian]

Dawes, B. (1960) A study of the miracidium of *Fasciola hepatica* and an account of the mode of penetration of the sporocyst into *Lymnaea truncatula*. In: *Lilro Hmenaje*, Dr Eduardo Caballero of Caballero, Escuela Nacional de Ciencias Biologicas, Mexico. pp. 95-111.

Dawes, B. (1962) On the growth and maturation of *Fasciola hepatica* in mouse. *Journal of Helminthology*, **36**: 11-18.

Dawes, B. and Hughes, D.I. (1964) Fascioliasis: The invasive stages of *Fasciola hepatica* in mammalian hosts. *Advances in Parasitology*, **2**: 97-168.

Dawkins, H.J.S., Windon, R.G. and Eagleson, G.K. (1989) Eosinophil responses in sheep selected for high and low responsiveness to *Trichostrongylus columbriformis*. *International Journal for Parasitology*, **19**: 199-205.

De Bruijin, M.H.L. (1988) Diagnostic DNA amplification: no respite for the elusive parasite. *Parasitology Today*, **4**: 293-295.

Degremont, A.A. (1973) Mangoky Project, Campaign against schistosomiasis in the lower Mangoky (Madagascar). Basle: Swiss Tropical Institute.

Dessein, A.J., Parker, W.L., James, S.L. and David, J.R. (1981) IgE antibody and resistance to infection. I.Selective suppression of IgE antibody response in rats diminishes the resistance and eosinophil response to *Trichinella spiralis* infection. *Journal of Experimental Medicine*, **153**: 423-436.

Dessouky, M.I. and Moustafa, H. (1978) Some haematological and biochemical studies on *Fasciola* infected buffaloes. *Assiut Veterinary Medicine Journal*, **3**: 177-187.

DFAMS (1990) *Agricultural Statistics of Nepal 1990*. Department of Food and Agriculture Marketing Services, Ministry of Agriculture, His Majesty's Government of Nepal.

Dhar, D.N., Bansal, G.G. and Sharma, R.L. (1985) Studies on the aquatic snails of Kashmir valley with particular reference to *Lymnaea auricularia sensu stricto*. *Indian Journal of Parasitology*, **9**: 241-244.

Dickson, K.E. (1964) The relative suitability of sheep and cattle as host for the liver fluke, *Fasciola hepatica* L. *Journal of Helminthology*, **38**: 203-212.

Dimatulac, C. and Pinto, L. (1983) Occurrence of *Chaetogaster limnaei* (Oligochaeta) in gastropod snail *Lymnaea philippinensis* Nevill in Metro Manila, and its possible role in the control of fascioliasis. *Philippine Journal of Veterinary Medicine*, **22**: 134-143.

Dinnen, J.K., Kelly, J.D. and Campbell, N.J. (1978) Further observations on the nature and characteristics of cross protection against *Fasciola hepatica* produced in sheep by prior infection with *Cysticercus tenuicollis*. *International Journal of Parasitology*, **8**: 173-176.

Dinnik, J.A. and Dinnik, N.N. (1957) A mud snail, *Lymnaea mweruensis* Connolly, as an intermediate host of both the liver flukes *Fasciola hepatica* L. and *Fasciola gigantica* Cobbold. Annual Report of East African Veterinary Research Organization, 1956-1957, Govt. Printer, Nairobi, Kenya.

Dinnik, J.A. and Dinnik, N.N. (1959) Effect of seasonal variations of temperature on the development of *Fasciola gigantica* eggs in the Kenya Highlands. *Bulletin of Epizootic Diseases of Africa*, **7**: 357-369.

Dinnik, J.A. and Dinnik, N.N. (1963) Effect on the seasonal variations of temperature on the development of *Fasciola gigantica* in the snail host in the Kenya Highlands. *Bulletin of Epizootic Diseases of Africa*, **11**: 197-207.

Dixon, K.E. (1964) The relative suitability of sheep and cattle as hosts for the liver fluke, *Fasciola hepatica* L. *Journal of Helminthology*, **38**: 203-212.

Doxey, D.L. (1983) The Liver. In: *Clinical Pathology and Diagnostic Procedures*, 2nd edn., pp. 47-52. Bailliere Tindall, London.

Doy, T.G. and Hughes, D.L. (1982) The role of the thymus in eosinophil response of rats infected with *Fasciola hepatica*. *Clinical and Experimental Immunology*, **47**: 74-76.

Dumag, U.P., Batalos, J.A., Escandor, N.B., Castillo, A.M. and Gajudo, C.E. (1976) The encystment of *Fasciola gigantica* metacercariae on different pasture grasses. *Philippines Journal of Animal Industries*, **31**(1-4): 72-86.

Duwel, D.S., Sambeth, W. and Bossaller, W. (1972) On the pathogenicity of *Fasciola hepatica* in sheep. *Parasitologia*, **14**: 35-44.

Edwards, C.M., al Saigh, M.N.R., Williams, G.L. and Chamberlain, A.G. (1976) Effect of liver fluke on wool production in Welsh mountain sheep. *Veterinary Record*, **98**: 372.

EL Samani, F., Mahmoud, O.M., Fawi, M.T., Gameel, A.A. and Haroun, E.M. (1985) Serum enzyme activity and bilirubin concentration in sheep experimentally infected with *Fasciola hepatica* in calves. *Journal of Comparative Pathology Pathology*, **95**: 499-503.

El-Harith, A. (1980) The influence of the alternative intermediate host '*Lymnaea truncatula*' on the development and pathogenesis of *Fasciola gigantica* infection in sheep. *Sudan Journal of Veterinary Science and Animal Husbandry*, **21**(1): 16-25.

Enigk, K, Feder, H., Dey Hazra, A. and Weigartner, E. (1972) Mineral content of blood plasma, erythrocytes, and liver during the prepatent period of fascioliasis. *Zentralblatt fur Veterinarmedizen Reihe B*, **19**: 239-257.

Enokihara, H., Furusawa, S., Nakakubo, H., Kajitani, H., Nagashima, S., Saito, K., Shishido, H., Hitoshi, K., Takatsu, K., Noma, T., Shimizu, A. and Honjo, T. (1989) T cells from eosinophilic patients produce Interleukin-5 with Interleukin-2 stimulation. *Blood*, **7**: 1809-1813.

Enzenauer, R.W. and Yamaoka, R.M. (1982) Eosinophilic meningitis and hydrocephalus in an infant. *Archives of Neurology*, **39**: 380-381.

Esclaire, F., Audousset, J.C., Rondelaud, D. and Dreyfuss, G. (1989) Floating metacercariae of *Fasciola hepatica* L.: some observations on their structure and quantitative variations during an experimental infection in *Lymnaea truncatula* Muller. *Bulletin de la Societe Francaise de Parasitologie*. **7**: 225-228. Seen as abstract, SilverPlatter 3.0, CAB Abstract 1990-1991.

Fagbemi, B.O. and Obarisiagbon, I.O. (1991) Common antigens of *Fasciola gigantica, Dicrocoelium hopses* and *Schistosoma bovis* and their relevance to serology. *Veterinary Quarterly*, **13**: 81-87.

Ford, E.J.H. (1965) Changes in the activity of ornithin carbamyl transferase (OCT) in the serum of cattle and sheep with hepatic lesions. *Journal of Comparative Pathology*, 75: 299-308.

Foreyt, W.F. (1982) The role of liver fluke in infertility of beef cattle. *Proceedings of 14th Annual Conference of American Association of Bovine Practitioners*. pp. 99-103.

Frandsen, F. and Christensen, N.O. (1984) An introductory guide to the identification of cercariae from African freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance. *Acta Tropica*, **41**: 181-202.

Fredriksson G., Kindahl, H. and Edqvist, L.E. (1985) Endotoxin induced prostaglandins release and corpus luteum function in goats. *Animal Reproduction Science*, **8**: 109-121.

Furmaga, S., Gundlach, J.L. and Sobieszewski, K. (1974) Studies on the behaviour of white blood cell systems in experimental fascioliasis in sheep and rabbits. In: *Proceedings of the 3rd International Congress of Parasitology*, 25-31 August, Munich, 1974, **1**: 497.

Furmaga, S. and Gundlach, J.L. (1978) Behaviour of morphotic blood elements and levels of iron and transfer in the blood serum of calves experimentally infected with *Fasciola hepatica* (Trematoda). *Acta Parasitologica Polonica*, **25**: 179-189.

Garate, T., Harnett, W. and Parkhouse, R.M.E. (1990) Cloning of a species-specific DNA probe from *Onchocerca gibsoni*. *International Journal for Parasitology*, **20**: 31-35.

Gatenby, R.M., Mahato, S.N. and Shrestha, N.P. (1992) Animal production in the hills of east Nepal: Potential for improvement. In: *Livestock Production and Diseases in the Tropics*, (eds. H. Kuil, R.W. Paling and J.E. Huhn), pp. 246-248. International Agricultural Centre, Wageningen, the Netherlands.

Gibson, W.C., Dukes, P. and Gashumba, J.K. (1988) Species-specific DNA probes for the identification of African trypanosomes in tsetse flies. *Parasitology*, **97**: 63-73.

Goodall, E.A., McIlroy, S.G., Stewart, D.A. and McCracken, R.M. (1989) An autoregressive multivatiate model for fasciolosis. In: Proceedings of a Meeting of Society for Veterinary Epidemiology and Preventive Medicine, (ed. G.J. Rowlands), pp. 1-12. University of Exeter.

Gupta, S.C., Prasad, A. and Chandra, R. (1986) Studies on the predatory behaviour of *Channa punctatus* towards *Lymnaea auricularia*, the intermediate host of *Fasciola gigantica*. *Indian Journal of Parasitology*, **10**: 123-125.

Guralp, N., Ozcan, C. and Simms, B.T. (1964) *Fasciola gigantica* and fascioliasis in Turkey. *American Journal of Veterinary Research*, **25**: 196-210.

Haiba, M. H., El-Rawii, K. A. and Osman, H. G. (1964) A comparative study on the levels of Ca, inorganic P and Mg in the blood serum and bile of the normal healthy buffalo (*Bos bubalus*) and those of buffaloes infested with liver fluke (*Fasciola gigantica*). *Zeitschrift fur Parasitenkunde*, **23**: 527-531.

Hammond, J.A. (1965) Observations on fascioliasis in Tanganyika. *Bulletin of Epizootic Diseases of Africa*, **13**: 55-65.

Hammond, J.A. (1970) Studies on fascioliasis with special reference to *Fasciola gigantica* in east Africa. University of Edinburgh, Ph.D. Thesis.

Hammond, J.A. (1972) Infections with *Fasciola* spp. in wild life in Africa. *Tropical Animal Health and Production*, **4**: 1-13.

Hammond, J.A. (1973) Experimental chronic *Fasciola gigantica* infection in sheep. *Tropical Animal Health and Production*, **5**: 12-21.

Hammond, J.A. and Sewell, M.M.H. (1974) The pathogenic effect of experimental infections with *Fasciola gigantica* in cattle. *British Veterinary Journal*, **130**: 453-465.

Hammond, J.A. and Sewell, M.M.H. (1975) Experimental infections of cattle with *Fasciola gigantica*: Numbers of parasites recovered after varying periods of infection. *Tropical Animal Health and Production*, **7**: 105-113.

Hammond, J.A. and Sewell, M.M.H. (1990) Diseases caused by helminths. In: *Handbook on Animal Diseases in the Tropics*, 4th edn., (eds. M.M.H. Sewell and D.W. Brocklesby), pp. 104-159. Bailliere Tindall.

Hanna, R.E.B. and Jura, W. (1976) *In vitro* maintenance of juvenile *Fasciola gigantica* and their use to establish infections in mice. *Research in Veterinary Science*, **21**: 244-246.

Harnett, W., Chambers, A.E., Renz, A. and Parkhouse, R.M.E. (1988) The use of synthetic oligonucleotide probes in the DNA identification of *Onchocerca volvulus*. *Molecular and Biochemical Parasitology*, **35**: 119-126.

Haroun, E.M. (1979) Studies on the resistance to *Fasciola hepatica* in rats and rabbits. Ph.D. Thesis. University of Edinburgh.

Haroun, E.M., Abdel Gadir, H. and Gameel, A.A. (1986) Studies on naturally occurring ovine fascioliasis in the Sudan. *Journal of Helminthology*, **60**: 47-53.

Haroun, E.M., El Sanhouri, A.A. and Gameel, A.A. (1988) Response of goats to repeated infections with *Fasciola gigantica*. *Veterinary Parasitology*, **30**: 287-296.

Harris, H. and Hopkinson, D.A. (1976) *Handbook of Enzyme Electrophoresis in Human Genetics*, 1st edn. North Holland Publishing Co., Oxford.

Harrison, L.J.S. (1991) Monoclonal antibodies and DNA probes in Diagnosis. In: *Biotechnology in Livestock Development in Developing Countries*, (ed. A.G. Hunter), pp. 254-271. Centre for Tropical Veterinary Medicine, University of Edinburgh.

Harrison, L.J.S., Delgado, J. and Parkhouse, R.M.E. (1988a) Differentiation of *Taenia saginata* and *Taenia solium* by use of cloned DNA fragments. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **82**: 174.

Harrison, L.J.S., Delgado, J. and Parkhouse, R.M.E. (1988b) DNA probes in the differentiation of human *Taenia saginata* and *Taenia solium* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **82**: 939.

Harrison, L.J.S., Delgado, J. and Parkhouse, R.M.E. (1990) Differential diagnosis of *Taenia saginata* and *Taenia solium* with DNA probe. *Parasitology*, **100**: 459-461.

Harvey, D.G. (1962) Intermediate metabolism of the carbohydrates. In: *Biochemistry for Veterinary Students*, 1st edn., pp. 168-173. Blackwell Scientific Publications, Oxford.

Hawkins, C.D. (1984) Productivity in sheep treated with diaphenathide at different times after infection with *Fasciola hepatica*. *Veterinary Parasitology*, **15**: 117-123.

Hawkins, C.D. and Morris, R.S. (1978) Depression of productivity in sheep infected with *Fasciola hepatica*. *Veterinary Parasitology*, **4**: 341-352.

Hayes, T.J. (1978) Further evidence for the early expression of immunity to *Fasciola hepatica* in rats. *Journal of Parasitology*, **64**: 374-376.

Hayes, T.J. and Mitrovic, M. (1977) The early expression of protective immunity to *Fasciola hepatica* in rats. *Journal of Parasitology*, **63**: 584-587.

Hillyer, G.V. (1980) Isolation of *Fasciola hepatica* tegument antigens. *Journal of Clinical Microbiology*, **12**: 695-699.

Hillyer, G.V. and Taylor, D.W. (1988) Immunoprecipitation of *Fasciola hepatica* mRNA *in vitro* translation products using infection and hyperimmune sera. *American Journal of Tropical Medicine and Hygiene*, **38**: 547-552.

Hillyer, G.V. and Soler de Galanes, M. (1991) Initial feasibility of the FAST-ELISA for the immunodiagnosis of fascioliasis. *Journal of Parasitology*, **77**: 362-365.

Holmes, P.H., Dargie, J.D., MacLean, J.M. and Mulligan, W. (1968a) The anaemia in fascioliasis: studies with 51Cr-labelled red cells. *Journal of Comparative Pathology*, **78**: 415-420.

Holmes, P.H., Dargie, J.D., MacLean, J.M. and Mulligan, W. (1968b) Albumin and globulin turnover in chronic ovine fascioliasis. *Veterinary Record*, **83**: 227-228.

Hope Cawdery, M.J. (1976) The effects of fascioliasis on ewe fertility. *British Veterinary Journal*, **132**: 568-575.

Hope Cawdery, M.J. (1984) Review of the economic importance of fasciolosis in sheep and cattle. *Iris Veterinary News*, **6**: 14-22.

Hope Cawdery, M.J., Strickland, K.L., Conway, A. and Crowe P.J. (1977) Production effects of liver fluke in cattle.1. The effects of infection on live weight gain, feed intake and food conversion efficiency in beef cattle. *British Veterinary Journal*, **133**: 145-159.

Horner, M.R. (1965a) The interpretation of faecal egg counts. I. Daily variation in *F. hepatica* egg counts in cattle. *Zeitschrift fur Parasitenkunde*, **26**: 143-155.

Horner, M.R. (1965b) The interpretation of faecal egg counts. II. Single and multiple sampling in the diagnosis of subclinical *F. hepatica*. *Zeitschrift fur Parasitenkunde*, **26**: 156-162.

Horner, M.R. (1965c) The interpretation of faecal egg counts. III. The influence of age of the host on *F. hepatica* egg counts in cattle. *Zeitschrift fur Parasitenkunde*, **26**: 221-229.

Horner, M.R. (1967) The interpretation of faecal egg counts. IV. The influence of faecal consistency and dry matter content on *F. hepatica* egg counts in cattle. *Zeitschrift fur Parasitenkunde*, **28**: 211-218.

Horschner, F., Hennings, R., Verspohl, F., Averbeck, W. and Boch, J. (1976) Chemotherapeutic control of fascioliasis of cattle in the Steinfurt area. II.Results after a 3-year period of treatment. *Berliner and Muenchner Tieraerztliche Wochenschrift*, **83**: 21-26.

Huang, S.W., Lai, T.S., Chen, L.J. and Shien, Y.S. (1979) Species of *Fasciola* prevalent among cattle in Taiwan. *Journal of Chinese Society of Veterinary Science*, **5**: 79-85.

Hubendick, B. (1951) Recent Lymnaeidae. *Kungl Svenska Vetenskaps Akademiens Handlingar*, **3**: 1-223.

Hughes, D.L. (1987) *Fasciola* and *Fascioloides*. In: *Immune Responses in Parasitic Infections: Immunology, Immunopathology and Immunoprophylaxis.* Trematodes and Cestodes (ed. E.J.L. Soulsby), Vol.II, pp. 91-114. CRC Press, Florida, USA.

Hughes, D.L., Treacher, R.J. and Harness, E. (1973) Plasma enzyme changes in goats infected with *Fasciola hepatica* and the effect of nitroxynil. *Research in Veterinary Science*, **15**: 249-255.

Hughes, D.L., Treacher, R.J. and Harness, E. (1974) The anthelmintic activity of diamphenethide against immature *Fasciola hepatica* in goats and the course of experimental infections as demonstrated by plasma enzyme changes. *Research in Veterinary Science*, **17**: 302-311.

Hughes, D.L., Harness, E. and Doy, T.G. (1977) Loss of ability to kill *Fasciola hepatica* in sensitised rats. *Nature* (London), **267**: 517-518.

Hughes, D.L., Harness, E. and Doy, T.G. (1978) Failure to demonstrate resistance in goat, sheep and cattle to *Fasciola hepatica* after infection with *Cysticercus tenuicollis*. *Research in Veterinary Science*, **25**: 356-359.

Hunter, R.W. (1953) On migration of *Lymnaea peregra* (Muller) on the shores of Loch Lomond. *Proceedings of Royal Society of Edinburgh*, **65**: 84-105.

Hussey, R.S. (1979) Biochemical systematics of nematodes - a review. *Helminthological Abstracts*, **48**: 141-148.

Irving, D.O. and Howell, M.J. (1981) Preparation and in vitro translation of mRNA from *Fasciola hepatica. Molecular and Biochemical Parasitology*, **4**: 337-348.

Isseroff, H., Spengler, R. N. and Charnock, D. R. (1979) Fascioliasis: similarities of the anaemia in rats to that produced by infused proline. *Journal of Parasitology*, **65**: 709-714.

Jablonowski, Z., Liminowicz, J. and Romanic, K. (1971) Effect of liver fluke infection on ascaridosis and the level of sodium, potassium and calcium in rat blood serum. *Wiad Parazyt.*, **17**:151-157.

Jackson, H.G. (1921) A revision of the genus *Fasciola*, with particular reference to *Fasciola gigantica* and *F. nyanzi* (Leiper). *Parasitology*, **13**: 48-56.

Jakubowski, M.S. and Barnard, D.E. (1971) Anaphylactic shock during operation for hydatid disease. *Anaesthesiology*, **34**: 197-199.

Janway Ltd. (1988) Determination of sodium and potassium in biological fluids. *Janway Application Advice No. 3*. Janway Ltd., Gransmore Green, Felsted, Dunmow, Essex, England.

Janzen, E.D., Orr, J.P. and Osborne, A.D. (1981) Bacillary haemoglobinuria associated with hepatic necrobacillosis in a yearling feedlot heifer. *Canadian Veterinary Journal*, **22**: 393-394.

Jarret, E.E.E. and Miller, H.R. (1982) Production and activities of IgE in helminth infection. *Progress in Allergy*, **31**: 178-233.

Jennings, F.W., Mulligan, W. and Urquhart, G.M. (1956) Radioisotope studies on the anaemia produced by infection with *Fasciola hepatica*. *Experimental Parasitology*, **V**: 458-468.

Joshi, B.R. (1986) Evaluation of rice-straw as a potential source for fasciola infection of the ruminants in Nepal. Lumle Agricultural Centre. Technical Paper No. **14/86**.

Joshi, B.R. (1987) Evaluation of carbon tetrachloride (CTC) drenching against fascioliasis in a pocket area of mid-western hills in Nepal. Technical Paper No. 87/15, Lumle Agricultural Centre, Kaski, Nepal.

Joshi, B.R. (1988) Prevalence of fascioliasis (liver fluke) in cattle and buffaloes in the mid-western hills of Nepal. *Journal of Institute of Agriculture and Animal Science*, **9**: 111-114.

Joshi, B.R. (1989) A study on rice straw feeding to evaluate its role in *Fasciola* (liver fluke) infection in Nepalese farm animals. In: *Livestock Production in the Tropics*, (eds. H. Kuil, R. W. Paling and J. E. Huhn). Proceedings of the 6th International Conference of Institutes for Tropical Veterinary Medicines, 28 Aug-1 Sept 1989, Wageningen, The Netherlands. pp. 354-357.

Joshi, D.D. and Tewari, H.C. (1975) Some observations on incidence of fascioliasis in yak and hilly cattle. *Bulletin of Veterinary Science and Animal Husbandry, Nepal*, **4**: 1-3.

Kadhim, J.K. (1976) Haematological changes during the course of experimental infection with *Fasciola gigantica* in sheep. In: *Pathophysiology of Parasitic Infections*, (ed. E.J.L. Soulsby), pp. 105-114. Academic Press, New York.

Kaneko, J.J. (editor) (1980) *Clinical Biochemistry of Domestic Animals*, 3rd edn., pp. 201-257. Academic Press, New York.

Kendall, S.B. (1949) Nutritional factors affecting the rate of development of *Fasciola hepatica* in *Lymnaea truncatula*. *Journal of Helminthology*, **53**: 179-190.

Kendall, S.B. (1950) Snail hosts of *Fasciola hepatica* in Britain. *Journal of Helminthology*, **24**: 63-74.

Kendall, S.B. (1953) The life history of *Lymnaea truncatula* under laboratory conditions. *Journal of Helminthology*, 27: 17-28.

Kendall, S.B. (1954) Fascioliasis in Pakistan. *Annals of Tropical Medicine and Parasitology*, **48**: 307-313.

Kendall, S.B. (1965) Relationship between the species of *Fasciola* and their molluscan hosts. *Advances in Parasitology*, **3**: 59-98.

Kendall, S.B. and McCullough, F.S. (1951) The emergence of the cercariae of *Fasciola hepatica* from the snail *Lymnaea truncatula*. *Journal of Helminthology*, **25**: 77-92.

Kendall, S.B. and Ollerenshaw, C.B. (1963) The effect of nutrition on the growth of *Fasciola hepatica* in its snail host. *Proceedings of Nutrition Society*, **22**: 41-46.

Kendall, S.B. and Parfitt, J.W. (1953) Life-history of *Fasciola gigantica* Cobbold 1856. *Nature*, **171**: 1164-1165.

Kendall, S.B. and Parfitt, J.W. (1959) Studies on the susceptibility of some species of *Lymnaea* to infection with *Fasciola gigantica* and *F. hepatica*. *Annals of Tropical Medicine and Parasitology*, **53**: 220-227.

Kendall, S.B. and Parfitt, J.W. (1965) The life-history of some vectors of *Fasciola gigantica* under laboratory conditions. *Annals of Tropical Medicine and Parasitology*, **59**: 10-16.

Kendall, S.B., Sinclair, I.J., Everett, G. and Parfitt, J.W. (1978) Resistance to *Fasciola hepatica* in cattle. I. Parasitological and serological observations. *Journal of Comparative Pathology*, **88**: 115-122.

Khalil, L.F. (1961) On the capture and destruction of miracidia by *Chaetogaster limnaei* (Oligochaeta), *Journal of Helminthology*, 35: 269-274.

Kimura, S. and Shimizu, A. (1978) Viability of F. gigantica metacercariae. *Japanese Journal of Veterinary Science*, **40**: 357-359.

Kimura, S., Shimizu, A. and Kawano, J. (1984) Morphological observation on liver fluke detected from naturally infected carabaos in the Philippines. Sci. Rep. Fac. Agr. Kobe Univ. **16**: 353-357.

Klein, J. (1990) *Immunology*. Blackwell Scientific Publications, London.

Klimenko, B.B. and Sazanov, A.M. (1972) A possibility of differentiation and identification of several trematode species and their intermediate hosts by disc electrophoresis method in polyacrylamide gel. *Vsesoyuznogo Instituta Gelmintologii*, **19**: 102-105

Kloos, H. and McCullough, F.S. (1982) Plant molluscicides. *Journal of Medicinal Plant Research*, **46**: 195-209.

Kumar, M., Maru, A. and Pachauri, S. P. (1982) Changes in blood cellular components, serum protein concentrations and serum enzyme activities in buffaloes infested with *Fasciola gigantica*. *Research in Veterinary Science*, **33**: 260-261.

Kumar, S. and Sharma, M.C. (1991) Infertility in rural cows in relation to fascioliasis. *Indian Journal of Animal Sciences*, **61**: 838-840.

Lang, B.Z. (1967) Host-parasite relationships of *Fasciola hepatica* in the white mouse. II. Studies on acquired immunity. *The Journal of Parasitology*, **53** (1): 21-30.

Lang, B.Z., Larsh, J.E., Weatherly, F.N. and Goulson, T.H. (1967) Demonstration of immunity to *Fasciola hepatica* in recipient mice given peritoneal exudate cells. *The Journal of Parasitology*, **53**: 208-209.

Lapage, G. (1968) Some trematoda parasitic in farm animals. In: *Veterinary Parasitology*, 2nd edn., pp. 329-245. Oliver and Boyd, Edinburgh.

Lee, C.G. and Zimmerman, G.L. (1993) Banding patterns of *Fasciola hepatica* and *Fasciola gigantica* (Trematoda) by isoelectric focusing. *Journal of Parasitology*, **79**: 120-123.

Lee, C.G., Zimmerman, G.L. and Wee, S.H. (1992) *Fasciola hepatica*: comparison of flukes from Korea and the United States by isoelectric focusing banding patterns of whole-body protein. *Veterinary Parasitology*, **42**: 311-316.

Lehner, R.P. (1977) In vitro culture of *Fasciola hepatica* and the immunology associated with the metabolic products of the trematode. Ph.D. Thesis. University of Edinburgh.

Lehner, R.P. and Sewell, M.M.H. (1980) A study of the antigens produced by adult *Fasciola hepatica* maintained in vitro. *Parasite Immunology*, **2**: 99-109.

Leimbacher, F. (1978) Experience with 'Mt' system of forecasting fascioliasis in France. In: Weather and Parasitic Disease. W.M.O. Technical Note No. 159. pp. 6-13.

Lohani, M.N. and Jaeckle, M.K. (1981/1982) An attempt to identify *Fasciola* species in Palpa. *Bulletin of Veterinary Science and Animal Husbandry, Nepal*, **10-11**: 1-7.

Losos, G.J. (1986) Fascioliasis (*Fasciola gigantica*). In: *Infectious Tropical Diseases of Domestic Animals*. pp. 882-902. Longman Scientific & Technical, Longman Group UK Limited, England.

Louden, S.A.I., McNeilly, A.S. and Milne, J.A. (1983) Nutrition and lactational control of fertility in red deer. *Nature* (London), **302**: 145-147.

Lowcock, M. (1982) Parasitological problems and anthelmintic usage in livestock in Nepal: a short survey and review. United Mission to Nepal.

LRMP (1986) Topographical Survey of Nepal. Land Resource Mapping Project, Babar Mahal, Kathmandu, Nepal.

Macan, T.T. (1974) Freshwater Ecology. 2nd edn. Longman, London.

Madsen, H. (1990) Biological methods for the control of freshwater snails. *Parasitology Today*, **6**: 237-241.

Madsen, H. and Frandsen, F. (1989) The spread of freshwater snails including those of medical and veterinary importance. *Acta Tropica*, 46: 139-146.

Mahato, S.N. (1984) Differentiation of *Fasciola* species and their intermediate hosts by enzyme electrophoresis. M.Sc. Dissertation. University of Edinburgh.

Mahato, S.N., Subba, D.B. and Tamang, P.M. (1989) Chemical composition of some tree fodders. Technical Report, Pakhribas Agricultural Centre, Dhankuta, Nepal.

Mahato, S.N., Rai, K. and Karki, I.B. (1991) Prevalence of fasciolosis in calves in Dhankuta district. *Veterinary Review*, **6**: 20-21.

Mahato, S.N. and Rai, K. (1992) Efficacy of triclabendazole against fasciolosis in buffaloes. *Veterinary Review*, **7**: 17-20.

Malek, E.A. (1980) Snail-Transmitted Parasitic Diseases, Vol.II. CRS Press, Boca Raton, Florida.

Malone, C.R. (1965) Dispersal of aquatic gastropods via the intestinal tract of water birds. *Nautilus*, **78**: 135-139.

Malone, J.B., Fehler, D.P., Loyacano,A.F. and Zukowski, S.H. (1992) Use of LANDSAT MSS imagery and soil type in a geographic information system to asses site-specific risk of fascioliasis on Red river basin farms in Louisiana. *Annals of the New York Academy of Science*, **653**: 389-397.

Malone, J.B., Loyacano, A., Armstrong, D.A. and Archbold, L.F. (1982) Bovine fascioliasis: economic impact and control in Gulf Coast based on seasonal transmission. *Bovine Practitioner*, **17**: 126-133.

Malone, J.B., Williams, T.E., Muller, R.A., Geaghan, J.P.and Loyacano, A.F. (1987) fascioliasis in cattle in Louisiana: Development of a system to predict disease risk by climate, using the Thornthwaite water budget. *American Journal of Veterinary Research*, **48**: 1167-1170.

Malviya, H.C. (1967) Morphological observations on *Fasciola gigantica* Cobbold, 1855, with remarks on the validity of *Fasciola indica* Varma, 1953. *Indian Journal of Veterinary Science*, **37**: 67-77.

Mango, A.M., Mango, C.K.A. and Esamal, D. (1972) A preliminary note on the susceptibility, prepatency and recovery of *Fasciola gigantica* in small laboratory animals. *Journal of Helminthology*, **46**: 381-386.

Matto, M.R., Bali, H.S. and Gupta, P.P. (1989) Pathological changes in the liver of buffaloes in fascioliasis and schistosomiasis. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, **10**: 176-179.

McCullough, F.S. (1981) Biological control of the snail intermediate hosts of human *Schistosoma* spp: a review of its present status and future prospects. *Acta Tropica*, **38**: 5-13.

McIlroy, S.G., Goodall, E.A., Stewart, D.A., Taylor, S.M. and McCracken, R.M. (1990) A computerised system for the accurate forecasting of the annual prevalence of fasciolosis. *Preventive Veterinary Medicine*, **9**: 27-35.

McLaren, D.J., Mackenzie, C.D. and Ramalho-Pinto, F.J. (1977) Ultrastructural observations on the *in vitro* interaction between rat eosinophils and some parasitic helminths (*Schistosoma mansoni, Trichinella spiralis* and *Nippostrongylus brasiliensis*). *Clinical and Experimental Immunology*, **30**: 105-118.

McManus, D.P. and Simpson, A.J.G. (1985) Identification of the *Echinococcus* (hydatid disease) organisms using cloned DNA markers. *Molecular and Biochemical Parasitology*, **17**: 171-178.

Meek, A.H. and Morris, R.S. (1981) A computer simulation model of ovine fasciolosis. *Agricultural Systems*, **7**: 49-77.

Milbourne, E.A. and Howell M.J. (1990) Eosinophil responses to *Fasciola hepatica* in rodents. *International Journal of Parasitology*, **20**: 705-708.

Mohsin, M., Rahman, M.M., Das, P.M. and Fazlul Haque, A.K.M. (1991) Haematological observations in cattle naturally infected with *Fasciola gigantica*. *Bangladesh Veterinarian*, **8**: 31-34.

Morel, A.M. and Mahato, S.N. (1987) Epidemiology of fascioliasis in the Koshi hills of Nepal. *Tropical Animal Health and Production*, **19**: 33-38.

Morel, A.M. (1987) Detection of *Fasciola gigantica* eggs in faeces: A comparision of methods. *Veterinary Record*, **120**: 90-91.

Morel, A.M. (1985) The prevalence of fascioliasis in cattle and buffalo in eastern hills of Nepal. Technical Paper No. 81, Pakhribas Agricultural Centre, Dhankuta, Nepal.

Moriyama, N., Tsuji, M. and Seto, T. (1979) Three karyotypes and their phenotypes of Japanese liver flukes (*Fasciola sp.*). *Japanese Journal of Parasitology*, **28**: 23-33. [In Japanese; English Summary].

Murray, M. and Rushton, B. (1975) The pathology of fascioliasis with particular reference to hepatic fibrosis. *Symposia of British Society for Parasitology*, **13**: 27-41.

Mzembe, S.A.T. and Chaudhary, M.A. (1979) The epidemiology of fascioliasis in Malawi 1. The epidemiology in the intermediate host. *Tropical Animal Health and Production*, **11**: 246-250.

Mzembe, S.A.T. and Chaudhary M.A. (1981) Epidemiology of fascioliasis in Malawi, Part II. Epidemiology in the definitive host. *Tropical Animal Health and Production*, **13**: 27-33.

Nadler, S.A. (1990) Molecular approach to studying helminth population genetics and phylogeny. *International Journal for Parasitology*, **20**: 11-29.

Nansen, P. (1975) Resistance in cattle to *Fasciola hepatica* induced by τ-ray attenuated larvae: results from a controlled field trial. *Research in Veterinary Science*, **19**: 278-283.

Nansen, P., Eriksen, L., Simensen, M.G. and Nielsen, K. (1968) Chronic fascioliasis in sheep. II. Metabolism of ^{131}I-labelled albumin and ^{125}I-labelled immunoglobulin-G. *Nordisk Veterinar Medicin*, **20**: 651-655.

Noorudin, M., Samad, M.A. and Rahman, A. (1982) A note on certain haematological and biochemical changes of Black Bengal goats infected with *Fasciola gigantica*. *Haryana Veterinarian*, **XXI**: 133-136.

Oakley, G.A., Owen, B. and Knapp, N.H.H. (1979) Production effects of subclinical liver fluke infection in growing dairy heifers. *Veterinary Record*, **104**: 503-507.

Ogunrinade, A.F. (1983) Bovine fascioliasis in Nigeria V. The pathogenicity of experimental infections in white Fulani cattle. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux*, **36**: 141-149.

Ogunrinade, A.F. (1984) Infectivity and pathogenicity of *Fasciola gigantica* in West African dwarf sheep and goats. *Tropical Animal Health and Production*, **16**: 161-166.

Ogunrinade, A.F. and Bamgboye, E.A. (1980) Bovine fascioliasis in Nigeria. I. Haematological indices and their correlation with worm burden in chronic fascioliasis. *British Veterinary Journal*, **136**: 457-462.

Ogunrinade, A.F. and Makinde, M.O. (1988) Observations on the pathogenesis of anaemia in fascioliasis and on immunoglobulin metabolism. In: *Nuclear Technique in the Study and Control of Parasitic Diseases of Livestock*, Proceedings of the final research co-ordination meeting on the use of nuclear techniques in the study and control of parasitic diseases of farm animals, Viana, Austria. pp. 139-148.

Ogunrinade, A.F., Adenaike, F.A., Fajimi, J.L. and Bamgboye, E.A. (1980) Bovine fascioliasis in Nigeria II. Blood chemistry and its correlation with burdens in chronic fascioliasis. *Zentralblatt fur Veterinarmedizen Reihe B*, **27**: 622-630.

Oldham, G. and Williams, L. (1985) Cell-mediated immunity to liver fluke antigens during experimental *Fasciola hepatica* infection in cattle. *Parasite Immunology*, **7**: 503-516.

Oli, K.P., Ratala, D.R. and Basnet, T.B. (1989) Initial endo-parasite surveillance in Naldung panchayat, Kavre district. *Veterinary Review*, **2**: 8.

Ollerenshaw, C.B. (1959) The ecology of the liver fluke (*Fasciola hepatica*). *Veterinary Record*, **71**: 957-963.

Ollerenshaw, C.B. (1966) The approach to forecasting the incidence of fascioliasis over England and Wales, 1958-1962. *Agricultural Meteorology*, **3**: 35-54.

Ollerenshaw, C.B. (1970) La prevision des maladies. Colloque organise al'Institute Noe, Rambouillet, P.17.

Ollerenshaw, C.B. (1973) Climate and forecasting liver fluke disease in Europe. In: *Helminth diseases of cattle, sheep and horses*, (eds. G.M. Urquhart and J. Armour).pp. 132-141. Proceedings of a Symposium held at the University of Glasgow. Spring 1973.

Ollerenshaw, C.B. (1974) Forecasting liver fluke disease. In: *The effects of Meteorological Factors Upon Parasites*. Symposium of the British Society for Parasitology, (eds. A.E.R. Taylor and R. Muller), Vol.12, pp.33-52. Blackwell Scientific Publications, Oxford, England.

Ollerenshaw, C.B. and Rowlands, W.T. (1959) A method of forecasting the incidence of fascioliasis in Anglesey. *Veterinary Record*, **71**: 591-598.

Ollerenshaw, C.B. and Smith, L.P. (1969) Meteorological factors and forecasts of helminth disease. *Advances in Parasitology*, **7**: 283-323.

Ollerenshaw, C.B. and Graham, E.G. (1986) Differentiation of the rediae of *Fasciola hepatica* and *Fasciola gigantica*. *Annals of Tropical Medicine and Parasitology*, **80**: 573-574.

Olsen, O.W. (1947) Longevity of metacercariae of *Fasciola hepatica* on pastures in the upper coastal region of Texas and its relationship to liver fluke control. *Journal of Parasitology*, **31**: 36-42.

Oostendorp, D. and Over, H.J. (1985) Production effects of liver fluke on cattle. *Top Rindvienhaltung*, **2**: 25-26.

Orr, J.P., Osborne, A.D. and Janzen, E.D. (1982) Calf bacillary haemoglobinuria in Saskatchewan. *Canadian Veterinary Journal*, **23**: 177-178.

Oshima, T., Akahane, H. and Shimazu, T. (1968) Patterns of the variations of the common liver fluke (*Fasciola* sp.) in Japan. I. Variations in the sizes and shapes of the worm and eggs. *Japanese Journal of Parasitology*, **17**: 97-105. [In Japanese; English summary].

Over, H.J. (1982) Ecological basis of parasite control: trematodes with special reference to fascioliasis. *Veterinary Parasitology*, **11**: 85-97.

Pachauri, S.P., Yadav, T.S. and Swarup, D. (1988) Studies on the epidemiology and economic impact of fascioliasis in goat. *International Journal of Animal Science*, **3**: 171-176.

Panaretto, B.A., Chapman, R.E., Downes, A.M., Reis, P.J. and Wallace, A.L.C. (1975) Some effects of three glucocorticoid analogues on wool growth and their efficacy as defleecing agent in sheep. *Australian Journal of Experimental Agriculture and Animal Husbandry*, **15**: 193-202.

Pantelouris, E.M. (1965) The common liver fluke. Pergamon Press, Oxford, England.

Parr, R.A., Cumming, I.A. and Clarke, I.J. (1982) Effects of maternal nutrition and plasma progesterone concentrations on survival and growth of the sheep in early gestation. *Journal of Agricultural Science Cambridge*, **98**: 39-46.

Paton, A. (1969) *Liver Diseases*. Heinemann Medical Books, London.

Perez, J.G., Vargas, M. and Malek, E.A. (1991) Displacement of *Biomphalaria glabrata* by *Thiara granifera* under natural conditions in the Dominican Republic. *Mem. Inst. Oswaldo Cruz. Rio de janeiro*, **86**: 341-347.

Prasad, A., Gupta, S.C. and Chandra, R. (1987) Lymnaeid snails as intermediate hosts of *Fasciola gigantica* in Rohilkhand and Kumaun region of Uttar Pradesh. *Indian Veterinary Journal*, **64**: 659-661.

Preston, J.M. and Castelino, J.B. (1977) A study of the epidemiology of bovine fascioliasis in Kenya and its control using N-Tritylmorpholin. *British Veterinary Journal*, **133**: 600-608.

Price, E.W. (1953) The fluke situation in American ruminants. *Journal of Parasitology*, **39**: 119-134.

Pritchard, D.I. and Eady, R.P. (1981) Eosinophilia in athymic nude (rnu/rnu) rat-thymus-independent eosinophilia? *Immunology*, **43**: 409-416.

Probert, A.J. and Lwin, T. (1977) *Fasciola hepatica*: the subcellular distribution and kinetic and electrophoretic properties of malate dehydrogenase. *Experimental Parasitology*, **41**: 89-94.

Probert, A.J. and Lwin, T. (1976) *Fasciola hepatica*: the presence of particle associated and soluble non-specific acid phosphatase. *Experimental Parasitology*, **40**: 206-211.

Pullan, N.B., Sewell, M.M.H. and Hammond, J.A. (1970) Studies on the pathogenicity of massive infections of *Fasciola hepatica* L. in lambs. *British Veterinary Journal*, **126**: 543-547.

Quiles, M.J., Lopez-Lopez, M.C., Hermoso, R., Monteoliva, M. and Lopez, M.C.L. (1985) Nucleic acids of *Fasciola hepatica* and *Dicrocoelium dendriticum* L. *Revista Iberica de Parasitologia*, **45**: 169-173.

Rahaman, K.A. and Ed Din, H.S. (1961) Observations on the distribution of drifting snails in a large irrigation canal. *Bulletin of World Health Organization*, **25**: 699-701.

Randell, W.F. and Bradley, R.E. (1980) Effects of hexachlorethane on the milk yields of dairy cows in North Florida infected with *Fasciola hepatica*. *American Journal of Veterinary Research*, **41**: 262-264.

Reid, J.F.S. and Armour, J. (1978) An economic appraisal of helminth parasites in sheep. *Veterinary Record*, **102**: 4-7.

Reid, J.F.S., Armour, J., Urquart, G.M. and Jennings, F.W. (1970) Studies on ovine fascioliasis. I. Observations on the sequential development of the naturally-acquired disease. *Veterinary Record*, **86**: 242-249.

Reid, J.F.S., Armour, J., Jennings, F.W., Kirkpatrick, K.S. and Urquhart G.M. (1967) The fascioliasis-ostertagiasis complex in young cattle. A guide to diagnosis and therapy. *Veterinary Record*, **80**: 371-374.

Reid, J.F.S., Dargie, J.D., Murray, M., Armour, J. and Over, H.J. (1973) Fascioliasis. In: *Helminth Diseases of Cattle, Sheep and Horses in Europe*, (eds. G.M. Urquhart and J. Armour), pp. 81-113. Robert MacLehose and Company Limited, Glasgow.

Reid, J.F.S., Doyle, J.J., Armour, J. and Jennings, F.W. (1972) *Fasciola hepatica* infection in cattle. *Veterinary Record*, **90**: 486-487.

Reinhard, E.G. (1957) Landmarks of parasitology. I. The discovery of the life cycle of the liver fluke. *Experimental Parasitology*, **6**: 208-232.

Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase. *Journal of Molecular Biology*, **113**: 237-251.

Rishi, A.K. and McManus, D.P. (1987) Genomic cloning of human *Echinococcus granulosus* DNA: isolation of recombinant plasmid and their use as genetic markers in strain characterization. *Parasitology*. **94**: 369-383.

Roberts, H.E. (1968) Observations on experimental acute fascioliasis in sheep. *British Veterinary Journal*, **124**: 433-449.

Roseby, F.B. (1970) The effect of fascioliasis on the wool production in merino sheep. *Australian Veterinary Journal*, **46**: 361-365.

Ross, J.G. (1970) The Stormont 'Wet Day' forecasting system for fasciolosis. *British Veterinary Journal*, **126**: 401-408.

Ross, J.G. (1978) Stormont 'Wet Day' fluke forecasting. In: Weather and Parasitic Animal Disease, pp. 14-20. WMO Technical Note No. 159.

Ross, J.G., Dow, C. and Todd, J.R. (1967) A study of *Fasciola hepatica* infections in sheep. *Veterinary Record*, **80**: 543-546.

Ross, J.G., Todd, G.R. and Dow, C. (1966) Single experimental infections of calves with the liver fluke, *Fasciola hepatica* (Linnaeus 1758). *Journal of Comparative Pathology*, **76**: 67-81.

Rowcliffe, S.A. and Ollerenshaw, C.B. (1960) Observations on the bionomics of the eggs of *Fasciola hepatica*. *Annals of Tropical Medicine and Parasitology*, **54**: 172-181.

Sahba, G.H., Arfaa, F., Farahmandian, I. and Jalali, H. (1972) Animal fascioliasis in Khuzestan, South-Western Iran. *Journal of Parasitology,* **58**: 712-716.

Sakaguchi, Y. (1980) Karyotype and gametogenesis of the common liver fluke, *Fasciola* sp. in Japan. *Japanese Journal of Parasitology*, **29**: 507-513.

Samad, M.A. and Haqub, M.E. (1987) Clinical occurrence of infectious necrotic hepatitis (Black Disease) in cattle of Bangladesh. *Indian Journal of Animal Health*,63-64.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989a) Molecular Cloning: A Laboratory Manual, 2nd edn, Vol. 3. Cold Spring Harbour Laboratory Press, New York.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989b) *Molecular Cloning: A Laboratory Manual*, 2nd edn, Vol. 2. Cold Spring Harbour Laboratory Press, New York.

Sanderson, C.J., Campbell, H.D. and Young, I.G. (1988) Molecular and cellular biology of eosinophil differentiation factor (Interleukin-5) and its effect on human and mouse B cells. *Immunological Reviews*, **102**: 29-50.

Santiago, N. and Hillyer, G.V. (1986) Isolation of potential serodiagnostic *Fasciola hepatica* antigens by electroelution from polyacrylamide gels. *American Journal of Tropical Medicine*, **35**: 1210-1217.

Sarwar, M.M. (1957) *Fasciola indica* Varma a synonym of *Fasciola gigantica* Cobbold. *Biologica*, **3**: 168-175.

Schillhorn van Veen, T.W. (1974) Drought: malnutrition and parasitism. *Nigerian Journal of Animal Production*, **1**: 231-236.

Schillhorn van Veen, T.W. (1979) Ovine fascioliasis (*Fasciola gigantica*) on the Ahmadu Bello University farm. *Tropical Animal Health and Production*, **11**: 151-156.

Schillhorn van Veen, T.W. (1980) Fascioliasis (*Fasciola gigantica*) in West Africa: a review. *The Veterinary Bulletin*, **50**(7): 529-533.

Sewell, M.M.H. (1962) The Immunology of fascioliasis with special reference to its relationship with the disease process. Ph.D. Thesis. University of Cambridge.

Sewell, M.M.H. (1964) The immunology of fascioliasis. II. Quantitative studies on the precipitation reaction. *Immunology*, **7**: 671-680.

Sewell, M.M.H. (1966) The pathogenesis of fascioliasis. Veterinary Record, **78**(3): 98-105.

Sewell, M.H.H. (1967) Serum enzyme activities in acute ovine fascioliasis. *Veterinary Record*, **80**: 577-578.

Sewell, M.M.H. (1976) The role of management in the control of helminth diseases. In: Beef Cattle Production in Developing countries, (ed. A.J. Smith), pp. 138-149. Lewis Reprints Ltd., Tonbridge.

Sewell, M.M.H. and Hammond, J.A. (1972) The detection of *Fasciola* eggs. *Veterinary Record*, **90**: 510-511.

Sewell, M.M.H., Hammond, J.A. and Dinning, D.C. (1968) Studies on the aetiology of anaemia in chronic fascioliasis in sheep. *British Veterinary Journal*, **124**: 160-170.

Sharma, R.L. and Bhat, T.K. (1989) Epidemiological and immunological studies on helminth infection in domestic animals and poultry in temperate agroclimatic regions of the country. In: Annual Report 1988-89, pp. 77. Indian Veterinary Research Institute, Izatnagar, UP, India.

Sharma, R.L., Dhar, D.N. and Raina O.K. (1989) Studies on the prevalence and laboratory transmission of fascioliasis in animals in the Kashmir Valley. *British Veterinary Journal*, **145**: 57-61.

Shiff, C.J. (1968) Location of *Bulinus* (Physópsis) *globosus* by miracidia of *Schistosoma haematobium*. *Journal of Parasitology*, **54**: 1133-1140.

Shiff, C.J. (1969) Influence of light and depth on location of *Bulinus* (Physopsis) *globosus* by miracidia of *Schistosoma haematobium*. *Journal of Parasitology*, **55**: 108-110.

Shiff, C.J. (1974) Seasonal factors influencing the location of *Bulinus* (Physopsis) *globosus* by miracidia of *Schistosoma haematobium* in nature. *Journal of Parasitology*, **60**: 578-583.

Shiramizu, K. and Abu, M. (1988) The preventive investigation of bovine fascioliasis. 4. The length of period of infectious ability of *Fasciola hepatica* metacercariae within straw bundle capsules preserved within a vinyl house or on the second floor of a barn. *Yamaguchi Journal of Veterinary Medicine*, **15**: 89-92.

Shrestha, E.K., Thakur, R.P., Dhakal, I.P. and Mahato, S.N. (1992) Prevalence and treatment of fasciolosis in cattle and buffaloes in Dhankuta district. *Veterinary Review*, **7**: 47-49.

Shubukin, C.D., White, M.W., Abrahamsen, M.S., Rognlie, M.C. and Knapp, S.E. (1992) A nulleic Acid-based test for detection of *Fasciola hepatica*. *Journal of Parasitology*, **78**: 817-821.

Sigma Diagnostics (1985) Phosphorus, inorganic: Quantitative, colorimetric determination in serum or urine at 620-700 nm (procedure No. 670). Sigma Diagnostics, St. Louis, USA.

Sigma Diagnostics (1987) Magnesium: Quantitative, colorimetric determination in serum or plasma at 520 nm (procedure No. 595). Sigma Diagnostics, St. Louis, USA.

Sigma Diagnostics (1988) Albumin: Quantitative, colorimetric determination of albumin in serum or plasma at 628 nm (procedure No. 631). Sigma Diagnostics, St. Louis, USA.

Sigma Diagnostics (1989) Total protein: Quantitative, colorimetric determination in serum or plasma at 540 nm (procedure No. 541). Sigma Diagnostics, St. Louis, USA.

Sigma Diagnostics (1990) Calcium: Quantitative, colorimetric determination in serum, plasma or urine at 575 nm (procedure No. 587). Sigma Diagnostics, St. Louis, USA.

Simpson, J.R., Kunkle, W., Courtney, Ch.H. and Shearer, J.K. (1985) Production losses and control of fascioliasis in cattle in Zimbabwe. *Agriculture Practice*, **6**: 20-24.

Sinclair, I.J. and Kendall, S.B. (1969) Precipitating antibodies to infection with Fasciola hepatica in rabbits. *Research in Veterinary Science*, **10**: 483-485.

Sinclair, K.B. (1962) Observations on the clinical pathology of ovine fascioliasis. *British Veterinary Journal*, **118**: 37-53.

Sinclair, K.B. (1964) Studies on the anaemia of ovine fascioliasis. *British Veterinary Journal*, **120**: 212-222.

Sinclair, K.B. (1965) Iron metabolism in ovine fascioliasis. *British Veterinary Journal*, **121**: 451-461.

Sinclair, K.B. (1966) The pathogenesis of fascioliasis. *Veterinary Record*, **78**: 106-109.

Sinclair, K.B. (1967) Pathogenesis of *Fasciola* and other liver flukes. *Helminthological Abstract*, **36**: 115-129.

Sinclair, K.B. (1969) Some aspects of the pathogenesis and treatment of fascioliasis. *Veterinary Record*, **84**: 544-547.

Sinclair, K.B. (1970) The effect of splenectomy on the pathogenicity of *Fasciola hepatica* in the sheep. *British Veterinary Journal*, **126**: 15-28.

Sinclair, K.B. (1972) The pathogenicity *Fasciola hepatica* in pregnant sheep. *British Veterinary Journal*, **128**: 249-259.

Singh, N.B., Basnyat, B.M., Eichenberger, G. and Bommeli, W. (1973) Report on Preparatory Phase of Parasite Control Project. HMG/SATA, Kathmandu, Nepal.

Singh, K.P. and Parihar, N.S. (1988) Pathology of fluke infestations in livers of sheep and goats. *Indian Journal of Animal Sciences*, **58**: 890-894.

Sinistin, D.F. (1933) Studien uber die Phylogenie der Trematoden. VI. The life histories of some American liver flukes. *Zeitschrift fur Parasitenkunde*, **6**: 170-191.

Smith, G. (1984a) Density-dependent mechanisms in the regulation of *Fasciola hepatica* population in sheep. *Parasitology*, **88**: 449-461.

Smith, G. (1984b) The impact of repeated doses of anthelmintic on intensity of infection and age structure of *Fasciola hepatica* populations in sheep. *Veterinary Parasitology*, **16**: 107-115.

Smith, G. (1987) The relationship between the density of *Fasciola hepatica* miracidia and the net rate of miracidial infections in *Lymnaea truncatula*. *Parasitology*, **95**: 159-163.

Sogoyan, I.S. (1956) Comparision of pathological changes caused in sheep by *Fasciola hepatica* and *F. gigantica*. *Trudy Armyanskogo Nauchno-issledovatelskogo Veternarnogo Instituta*, **1**: 113-117. [In Russian]

Soulsby, E.J.L. (1965) Textbook of Veterinary Clinical Parasitology. Vol.1, Helminths. Blackwell Scientific Publications, Oxford.

Soulsby, E.J.L. (1982) Helminths, Arthropods and Protozoa of Domesticated Animals. 7th edition. Bailliere, Tindall and Cassel, London.

Standen, O.D. (1963) Chemotherapy of helminth infections. In: Experimental Chemotherapy, (eds. R.J. Schnitzer and F. Hawking). pp. 701-892. Academic Press, New York and London.

Stephenson, W. (1947) Physiological and histological observations on the adult liver fluke, *Fasciola hepatica* L. II.Feeding. *Parasitology*, **38**: 123.

Suhardono, Widjajanti, S., Stevenson, P. and I.H. Carmichael (1991) Control of *Fasciola gigantica* with triclabendazole in Indonesian cattle. *Tropical Animal Health and Production*, **23**: 217-220.

Sukhapesna, V. (1992) Parasites of swamp buffaloes. In: *Buffalo Production*, (eds. N.M. Tulloh and J.H.G. Holmes), World Animal Science, C6, pp. 329-354. Elsevier, Amsterdam.

Swarup, D. and Pachauri, S.P. (1987a) Epidemiological studies on fascioliasis due to *Fasciola gigantica* in buffalo in India. *Buffalo Bulletin*, **6**: 4-9.

Swarup, D. and Pachauri, S.P. (1987b) Pathophysiology of fascioliasis in buffaloes: Some biochemical indices. *Indian Journal of Animal Sciences*, **57**: 1083-1085.

Swarup, D. and Pachauri, S.P. (1987c) Biochemical, histopathological and histochemical changes in liver of buffaloes affected by fascioliasis. *Indian Journal of Animal Sciences*, **57**: 1077-1082.

Swarup, D., Pachauri, S.P. and Mukherjee, S.C. (1987) Prevalence and clinicopathology of naturally occurring fascioliasis and biliary amphistomiasis in buffaloes. *Indian Journal of Animal Sciences*, **57**: 252-256.

Swarup, D., Pachauri, S.P. and Sharma, B. (1987) Comparative efficacy of serodiagnostic tests in buffaloes naturally infected with *Fasciola gigantica*. *Indian Journal of Animal Sciences*, **57**: 373-376.

Sykes, A.R., Coop, R.L. and Robinson, M.G. (1980) Chronic sub-clinical ovine fascioliasis: plasma glutamate dehydrogenase, gamma-glutamyl transpeptidase and aspartate aminotransferase activities and their significance as diagnostic aids. *Research in Veterinary Science*, **28**: 71-75.

Sykes, A.R., Coop, R.L. and Rushton, B. (1980) Chronic sub-clinical ovine fascioliasis: effects on food intake and food utilisation and blood constituents. *Research in Veterinary Science*, **28**: 63-70.

Symons L.E.A. (1985) Anorexia: Occurrence pathophysiology and possible causes in parasitic infections. *Advances in Parasitology*, **24**: 103-133.

Tandon, R.S. (1956) On *Fasciola indica* Varma, 1953, the common liver fluke of India. *Proceedings of the National Academy of Science (India), Allahabad*, **26**: 373-395.

Taylor, E.L. (1964) Fascioliasis and the Liver Fluke. FAO Agricultural Studies No. 64. FAO, Rome.

Ternopol'skaya, L.D. (1984) Variability of *Fasciola hepatica* L. 1758 in different hosts. *Bulletin Vsesoyuznogo Instituta Gelmintologie*, **38**: 47-51.

Thakuri, K.C. and Mahato, S.N. (1990) Prevalence of gastro-intestinal helminth infections in ruminants in Dhankuta district. In: *Livestock in the Hills of Nepal-2*, (eds. R.M. Gatenby, N.P. Shrestha and B.R, Thapa), P.120. Pakhribas Agricultural Centre, Dhankuta, Nepal.

Tinar, R. (1984) Differentiation of *Fasciola gigantica* and *Fasciola hepatica* according to the dimensions of their eggs. *Veteriner Fakultesi Dargise Ankara University*, **31**: 207-229.

Todd, J.R. and Ross, J.G. (1966) Origin of haemoglobin in the caecal contents of *Fasciola hepatica*. *Experimental Parasitology*, **19**: 151-154.

Tongson, M.S. (1978) A national fascioliasis control programme for the Philippines. *The Philippine Journal of Veterinary Medicine*, **17**: 106-120.

Trawford, A.F. (1988) Techniques for the differentiation of *Fasciola* species. M.Sc. Dissertation. University of Edinburgh.

Troncy, P.M. (1989) Helminths of livestock and poultry in tropical Africa. In: *Manual of Tropical Veterinary Parasitology*, English Edition, pp.3-175. CAB International, Wallingford, Oxon, England.

Troncy, P.M., Graber, M. and Thal, J. (1973) Phacochoerus aethiopicus (Pallas), a new host of *Fasciola gigantica* Cobbold, 1855. *Bulletin of Pathological Experiment*, **66**: 129-133.

Trudgett, A., Anderson, A. and Hanna, R.E.B. (1988) Use of immunosorbent-purified antigens of *Fasciola hepatica* in enzyme immunoassays. *Research in Veterinary Science*, **44**: 262-263.

Upatham, E.S. (1972a) Effects of some physico-chemical factors on the infection of *Biomphalaria glabrata* (Say) by miracidia of *Schistosoma mansoni* Sambon in St. Lucia, West Indies. *Journal of Helminthology*, **46**: 307-315.

Upatham, E.S. (1972b) Effects of water depth on the infection of *Biomphalaria glabrata* by miracidia of St. Lucian *Schistosoma mansoni* under laboratory and field conditions. *Journal of Helminthology*, **46**: 317-325.

Urquhart, G.M. (1954) Rabbit as host in experimental fascioliasis. *Experimental Parasitology*, **3**: 38-44.

Van Someren, V.D. (1946) The habitats and tolerance ranges of *Lymnaea* (*Radix*) *caillaudi*, the intermediate snail host of liver fluke in East Africa. *Journal of Animal Ecology*, **51**(2): 170-197.

Van Tiggle, L.J. and Over, H.J. (1976) Serological diagnosis of fascioliasis. *Veterinary Parasitology*, **1**: 239-248.

Varma, A.K. (1953) On *Fasciola indica* n. sp. with some observations on *Fasciola hepatica* and *Fasciola gigantica*. *Journal of Helminthology*, **27**: 185-198.

Walker, J. (1978) The finding of *Biomphalaria stramienea* amongst fish imported into Australia. WHO/SCHISTO/78: 46.

Walls, R.S. (1976) Lymphocytes and specificity of eosinophilia. *South African Medical Journal*, **50**: 1313-1318.

Walls, R.S. and Beeson, P.B. (1972) Mechanism of eosinophilia. 8.Importance of local cellular reactions in stimulating eosinophil production. *Clinical and Experimental Immunology*, **12**: 111-119.

Walls, R.S., Bass, D.A. and Beeson, P.B. (1974) Mechanism of eosinophilia. X.Evidence for immunologic specificity of stimulus. *Proceedings of the Society for Experimental Biology and Medicine*, **145**: 1240-1242.

Wamae, L.W. and Cheruiyot, H.K. (1990) Incidence of *Fasciola gigantica* intramolluscan stages in *Lymnaea natalensis*, in intermediate host, over a one-year period in Kenya. *Bulletin of Animal Health and Production in Africa.*, **38**: 5-6

Watanabe, S. (1962) Fascioliasis of ruminants in Japan. *Bulletin of Internal Epizootiology*, **58**: 313-322.

Watanabe, S. (1965) A revision of genus Fasciola in Japan, with particular reference to *F. hepatica* and *F. gigantica*. In: *Progress of Medical Parasitology in Japan*, vol. 2, (eds K. Morishita, Y. Komia and H. Matsubayashi), pp. 359-381. Meguro Parasitological Museum, Tokyo.

Watanabe, S. (1967) Fascioliasis of ruminants in Japan. *Japanese Agricultural Research Quarterly*, **2**: 22-27.

Weinbren, B.M. and Coyle, T.J. (1960) Uganda zebu cattle naturally infected with *Fasciola gigantica* with special reference to changes in the serum proteins. *Journal of Comparative Pathology*, **70**: 176-181.

West, H.J. (1991) Evaluation of total serum bile acid for diagnosis of hepatobiliary disease in cattle. *Research in Veterinary Science*, **51**: 133-140.

Wiedosari, E. and Copeman, D.B. (1990) High resistance to experimental infection with *Fasciola gigantica* in Javanese thin-tailed sheep. *Veterinary Parasitology*, **37**: 101-111.

Wilson, R.A. and Denison, J. (1970) Studies on the activity of the miracidium of the common liver fluke, *Fasciola hepatica*. *Comparative Biochemistry and Physiology*, **32**: 301-313.

Wilson, R.A. and Taylor, S.L. (1978) The effect of variations in host and parasite density on the level of parasitization of *Lymnaea truncatula* by *Fasciola hepatica*. *Parasitology*, **76**: 91-98.

Yadav, S.C. and Gupta, S.C. (1988) On the viability of *Fasciola gigantica* metacercariae ingested by *Lymnaea auricularia*. *Journal of Helminthology*, **62**: 303-304.

Yagi, A.I., Younis, S.A., Haroun, E.M., Gameel, A.A., Bushara, H.O. and Taylor, M.G. (1986) Studies on heterologous resistance between *Schistosoma bovis* and *Fasciola gigantica*. *Journal of Helminthology*, **60**: 55-59.

Yap, K.W. and Thompson, R.C.A. (1987) CTAB Preparation of cestode DNA. *Parasitology Today*, **3**: 220-222.

Yoshimura, K. (1989) The eosinophil in parasitic infections. In: Current Concepts in Parasitology, (ed.R.C. Ko), pp. 7-46. Hong Kong University Press, Hong Kong.

Younis, S.A., Yagi, A.I., Haroun, E.M., Gameel, A.A., Taylor, M.G. (1986) Immunization of zebu calves against *Fasciola gigantica*, using irradiated metacercariae. *Journal of Helminthology*, **60**: 123-134.

Zdun, V.I. and Yavorskii, I.P. (1984) Some aspects of the morphology of *Fasciola hepatica* varieties. *Materialy Nauchoi Konferentsii Vsesoyuznogo Obshchestva Gelmintologov*, **34**: 16-23.

Zukowski, S.N., Hill, J.M., Jones, F.W. and Malone J.B. (1991) Development and validation of a soil-based geographic information system model of habitat of *Fossaria bulimoides*, a snail intermediate host of *Fasciola hepatica*. *Preventive Veterinary Medicine*, **11**: 221-228.

Zurita, M., Bieber, D. and Mansour, T.E. (1989) Identification, expression and in situ hybridization of an eggshell protein gene from *Fasciola hepatica*. *Molecular and Biochemical Parasitology*, **37**: 11-18.

Zurita, M., Bieber, D., Ringold, G. and Mansour, T.E. (1987) Cloning and characterization of a female genital complex cDNA from the liver fluke, *Fasciola hepatica*. *Proceedings of the National Academy of Science of the USA*, **84**: 2340-2344.

APPENDICES

Appendix Table 3.1
Meteorological Record
15 Years Average (1977-1991)

Station: Pakhribas Agricultural Centre
Elevation: 1667m
Latitude: 27° 17'
Longitude: 87° 17'
Recording Time: 0840 & 1740

Month	Max Temp (°C)	Min Temp (°C)	R.H. (%)	Total Rainfall (mm)	Wet Days (No)	Sunshine (hr)	Evaporation (mm)	Wind speed (km/hr)
January	14.4	4.9	71	9	2	6.8	1.5	4.1
February	16.0	6.1	71	19	2	6.9	2.1	3.3
March	20.2	9.7	63	30	4	7.7	3.3	4.9
April	23.4	12.5	64	57	6	7.4	3.7	5.3
May	23.8	14.7	79	139	13	6.2	3.7	5.6
June	23.8	16.8	85	245	20	2.9	3.9	5.1
July	22.9	17.3	91	427	24	1.5	2.2	3.2
August	23.5	17.4	88	349	22	2.3	2.1	3.7
September	22.8	16.1	88	210	16	2.7	2.0	3.2
October	21.8	12.9	81	67	5	5.7	2.1	3.5
November	18.9	9.3	76	11	1	7.2	1.9	4.7
December	15.5	6.3	70	15	1	7.3	1.6	3.8

Station: Tarahara Agricultural Station
Elevation: 200m
Latitude: 26° 45'
Longitude: 87° 25'
Recording Time: 0840 & 1740

Month	Max Temp (°C)	Min Temp (°C)	R.H. (%)	Total Rainfall (mm)	Wet Days (No)	Sunshine (hr)	Evaporation (mm)	Wind speed (km/hr)
January	23.0	10.6	92.5	13.0	1.1	NA	NA	NA
February	25.3	12.5	86.1	27.1	3.1	NA	NA	NA
March	29.4	17.7	69.3	29.5	3.5	NA	NA	NA
April	32.9	21.5	59.5	68.4	5.7	NA	NA	NA
May	33.0	24.3	73.8	220.9	12.9	NA	NA	NA
June	32.9	25.5	79.5	327.6	16.1	NA	NA	NA
July	32.0	25.3	64.1	533.9	23.2	NA	NA	NA
August	32.4	25.6	84.1	451.2	21.9	NA	NA	NA
September	31.9	24.9	83.6	406.3	16.7	NA	NA	NA
October	31.5	22.4	79.9	55.0	5.2	NA	NA	NA
November	29.6	16.9	80.4	4.4	0.5	NA	NA	NA
December	25.7	12.4	89.8	9.9	0.9	NA	NA	NA

Note: NA = Not available

Appendix Table 4.1
Meteorological Record
(1991-1992)

Station: Pakhribas Agricultural Centre

Month	Maximum Temperature (°C)		Minimum Temperature (°C)		Relative humidity (%)		Rainfall	Wet Days
	Range	Mean	Range	Mean	Range	Mean	(mm)	(No)
January	08-18	12.9	01-09	5.3	56-92	79	51.2	3
February	12-21	16.5	06-13	8.9	52-94	73	4.0	1
March	10-25	21.2	07-14	10.6	47-85	67	16.4	4
April	18-28	24.4	10-17	12.7	35-92	67	22.1	5
May	20-27	23.1	12-17	14.2	62-94	81	155.5	18
June	22-26	23.3	14-19	16.6	82-99	92	328.6	22
July	21-26	24.0	16-21	18.0	76-100	91	256.8	26
August	21-26	23.5	15-19	17.4	83-100	92	299.6	26
September	21-24	22.7	10-17	15.8	86-100	93	469.9	19
October	20-25	21.7	10-16	13.1	67-93	84	0.9	1
November	15-22	18.7	08-15	11.5	60-94	79	0.0	0
December	10-19	15.3	0.5-09	5.8	43-91	70	15.1	3
January	11-17	14.1	00-09	4.4	61-94	78	1.5	1
February	10-16	13.9	2.5-09	4.8	74-100	86	18.5	4
March	16-29	21.9	07-16	10.7	58-90	74	0.0	0
April	21-31	26.1	07-18	13.8	40-96	79	20.3	4
May	21-2[illegible]	24.2	09-17	13.9	66-93	85	90.4	11

Station: Tarahara Agricultural Station

Month	Maximum Temperature (°C)		Minimum Temperature (°C)		Relative humidity (%)		Rainfall	Wet Days
	Range	Mean	Range	Mean	Range	Mean	(mm)	(No)
January	19-25	28.5	07-14	10.1	86-98	92	64.5	2
February	25-2[illegible]	27.1	10-17	13.0	72-100	84	5.0	1
March	26-35	31.0	15-25	18.2	57-90	70	35.8	3
April	30-3[illegible]	32.9	18-26	22.0	40-91	61	57.4	3
May	30-3[illegible]	32.3	20-27	24.8	54-95	71	108.4	8
June	29-34	32.2	23-28	25.4	65-100	82	402.6	21
July	28-35	32.2	24-28	26.2	68-95	81	322.0	21
August	31-3[illegible]	32.5	24-29	26.3	68-95	84	574.6	18
September	28-34	32.0	22-26	24.3	75-95	87	555.3	19
October	30-34	32.2	20-28	22.5	65-95	81	51.5	3
November	25-3[illegible]	29.0	13-21	16.0	60-95	81	0.0	0
December	17-2[illegible]	24.4	07-16	10.9	54-100	90	13.3	3
January	21-27	23.2	07-15	10.7	82-100	95	0.5	1
February	1[illegible]-2[illegible]	23.2	08-17	11.4	70-100	89	4.3	1
March	27-3[illegible]	30.8	13-24	18.0	45-90	60	0.0	0
April	30-4[illegible]	35.2	18-27	23.0	35-82	56	5.5	1
May	27-34	30.9	20-28	23.6	52-95	[illegible]	206.0	14

Appendix Table 4.2.1
Cross-sectional Survey
Snail habitats in the hills (January-February 1991)

H No.	Elevation (m)	Location	Type of Habitat	Snail densities (5 man min)	Snail species	Water quality: Colour/ turbidity	pH	Temp (°C)	EC (µs)	DO2 (mg/l)	Vegetation in habitat	Soil type
1	1475	Muga	RFSp	5	Larf	trans	7.0	6.0	35.7	0.8	algae, aquatic weed,azola,	loam
2	1500	Muga	RFSp	145	Larf, Last	trans	6.7	6.0	21.5	2.3	algae, aquatic weed,azola,	loam
3	1475	Muga	Pool	0		trans	6.4	10.9	38.5	0.2	algae, kane	loam
4	1450	Muga	RFSp	127	Larf	trans	6.5	16.5	58.0	0.7	watercress, aqutic weed,azola,algae	clay
5	1450	Muga	Pool	0		trans	6.5	12.0	39.0	0.4		loam
6	1450	Muga	RFSt	149	Larf, Last	trans	7.2	18.8	60.0	0.2	water cress, aquatic weed, azola	loam
7	1500	Muga	RFSt	4	Larf	trans	6.5	15.0	28.0	1.1	algae, azola, aquatic weed	clay
8	1400	Muga	RFSp	79	Larf	trans	6.5	6.5	58.0	0.8	azola, aquatic weed	loam
9	1400	Muga	RFSt	7	Larf	trans	7.0	11.5	48.0	0.5	algae, azola, aquatic weed	loam
10	1400	Muga	Stream	0		trans	6.8	12.3	33.8	1.4	algae	sandy
11	1400	Muga	Pool	6	Larf	trans	6.5	11.0	37.8	1.4	algae, aquatic weed,	sandy
12	1400	Muga	RFSp	102	Lv, Larf	trans	6.0	7.8	50.0	4.0	algae, azola, aquatic weed	loam
13	1400	Muga	IC	150	Lv, Larf	trans	7.2	10.5	36.2	1.7	algae	loam
14	1300	Muga	RFSp	7	Larf	trans	7.6	18.6	43.0	2.1	algae,azola,aquatic weed	loam
15	1300	Muga	RFSp	12	Larf	trans	7.8	22.7	22.3	1.7	watercress,algae,azola	clay
16	1450	Muga	RFSp	15	Larf	red	7.0	11.4	42.5	3.0	watercress,algae,azola	loam
17	1450	Muga	RFSp	98	Larf	trans	8.6	15.7	40.6	1.7	algae,rice	loam
18	1400	Muga	RFSt	0		dirty,red	6.4	14.0	52.0	0.9	aquatic weed,rice,calamus spp	sandy
19	1400	Muga	RFSp	17	Last	trans	7.7	15.7	22.7	1.6	azola,aquatic weed,algae	clay
20	1400	Muga	Pool	0		trans	6.7	12.8	38.8	1.5	calamus,aquatic weed,kane,algae	clay
21	1475	Phalate	SSt	0		cloudy	6.0	8.0	50.0	1.0	aquatic weed,water hyacynth	clay
22	1425	Muga	RFSt	6	Larf	turbid	6.3	16.0	86.0	0.8	watercress,azola,algae	loam
23	1350	Phalate	RFSt	12	Ll	trans	6.0	6.5	31.0	2.3	aquatc weed,algae	clay
24	1400	Phalate	RFSp	14	Larf, Lv	turbid	6.2	11.0	27.0	1.5	aquatic weed	clay
25	1400	Phalate	Pool	0		cloudy	6.5	9.9	48.0	2.0		sandy
26	1400	Phalate	RFSt	113	Larf	trans	6.7	12.9	82.0	2.0	azola	loam
27	1400	Phalate	RFSt	10	Larf	trans	6.6	14.0	47.0	1.9	azola,aquatic weed	loam
28	1350	Phalate	SSt	0		trans	6.2	13.0	46.0	0.7		clay
29	1300	Phalate	RFSt	5	Larf	turbid	6.4	14.2	42.1	0.6	aquatic weed	clay
30	1300	Phalate	RFSp	32	Larf, Ll, Lv	trans	6.8	12.4	48.0	1.6	aquatic weed,azola,algae	loam
31	1300	Phalate	RFSp	24	Larf	trans	6.6	8.2	82.7	1.2	algae,aquatic weed	loam
32	1400	Phalate	RFSp	38	Lv	trans	6.7	11.2	88.0	1.4	algae,aquatic weed,calamus spp	clay
33	1350	Phalate	RFSp	85	larf, Ll	trans	6.0	17.0	89.2	0.6	aquatic weed,algae	loam
34	1350	Phalate	RFSp	148	Larf, Ll	trans	6.1	18.3	64.8	1.2	algae,azola,aquatic weed	loam
35	1350	Phalate	Stream	0		trans	6.0	13.0	51.5	0.4		rocky
36	1350	Phalate	SSt	7	Larf, Ll	trans	6.5	14.2	83.0	1.1	azola,algae,aquatic weed	loam
37	1350	Phalate	RFSp	9	Ll	trans	6.5	14.6	84.4	3.0	algae,azola,aquatic weed	clay
38	1350	Phalate	RFSp	16	Larf	trans	6.7	19.8	24.0	2.1	algae,azola,aquatic weed	clay
39	1350	Phalate	RFSp	78	Larf	trans	6.1	18.7	77.0	1.4	algae,azola,aquatic weed	loam
40	1300	Phalate	RFSp	212	Larf, Lv	trans	7.0	9.8	61.4	2.1	algae,azola,aquatic weed	clay
41	1300	Phalate	RFSp	6	Larf	trans	6.8	11.6	46.0	0.6	algae,azola,aquatic weed	clay
42	1300	Phalate	RFSp	68	Lv, Ll	trans	6.2	14.1	60.2	1.5	algae,azola,aquatic weed	clay
43	1300	Phalate	RFSp	9	Larf	trans	6.2	12.0	48.0	1.3	algae,azola,aquatic weed	loam
44	1250	Phalate	RFSp	28	Ll, Lv	trans	6.9	12.5	23.5	1.6	algae,azola,aquatic weed	clay
45	1550	Phalate	RFSp	6	Larf	cloudy	6.6	19.7	84.2	0.7	algae,azola,aquatic weed	loam
46	1350	Phalate	RFSp	185	Larf, Lv	trans	6.8	17.6	74.0	0.6	algae,azola,aquatic weed,pig weed	loam
47	1150	Phalate	RFSp	165	Larf	trans	6.4	15.7	49.1	1.5	algae,azola,aquatic weed,pig weed	clay
48	1250	Phalate	RFSp	6	Lv	trans	7.4	10.6	44.8	2.0	algae	clay
49	1200	Phalate	Stream	0		trans	6.9	14.2	50.0	1.7	aquatic weed	loam
50	1200	Phalate	RFSp	187	Lv	trans	7.3	11.2	54.5	1.3	algae,azola,aquatic weed,kanchhi jhar	clay
51	1150	Phalate	RFSt	345	Lv, Larf	trans	7.8	14.0	68.7	1.8	algae,azola,aquatic weed	loam
52	1150	Phalate	RFSp	1	Ll	trans	7.2	11.4	43.0	2.7	algae,azola,aquatic weed	clay
53	1150	Phalate	RFSp	6	Larf	trans	7.3	11.5	45.0	0.9	algae,azola,aquatic weed	clay
54	1150	Phalate	RFSp	78	Larf	trans	7.2	12.1	48.0	1.0	algae,azola,aquatic weed	clay
55	1100	Phalate	RFSp	15	Lv, Ll	trans	7.0	16.0	112.0	1.7	algae,azola,aquatic weed,kanchhi jhar	loam
56	1100	Phalate	RFSp	2	Lv	trans	6.8	13.0	69.3	1.4	algae,azola,aquatic weed	loam
57	1150	Phalate	stream	0		trans	7.1	13.7	45.8	1.1	colocasia, kane	sandy
58	1250	Phalate	PoolSt	0		turbid	7.5	10.1	59.0	2.0		sandy
59	1200	Phalate	PoolSt	0		trans	7.8	10.2	38.3	1.8	algae	sandy
60	1200	Phalate	RFSp	18	Larf	trans	7.5	9.8	52.8	2.0	aquatic weed, algae	loam
61	1100	Muga	RFSt	201	Lv	trans	8.5	10.7	48.0	2.5	aquatic weed, algae	clay
62	1100	Muga	RFSp	204	Lv	trans	7.2	19.2	74.4	0.2	algae,azola,aquatic weed,kanchhi jhar	loam
63	1100	Muga	IC	2	Lv	trans	7.7	15.3	55.2	2.3	algae	clay
64	1100	Muga	RFSp	112	Lv, Ll	trans	7.8	15.7	49.0	1.3	aquatic weed	loam
65	1125	Muga	RFSp	4	Lv, Ll	trans	7.5	18.2	41.0	1.3	algae,azola,aquatic weed	clay
66	1100	Muga	RFSp	12	Lv, Ll	trans	7.8	20.5	49.7	2.0		sandy
67	900	Muga	RFSp	75	Ll	trans	8.8	25.9	52.5	2.9	algae,azola,aquatic weed	loam
68	875	Muga	RFSp	9	Ll	trans	6.9	23.7	61.2	2.9	algae,azola,aquatic weed	loam
69	850	Muga	RFSp	12	Ll, Larf	trans	8.1	25.6	51.0	2.9	algae,azola,aquatic weed	loam
70	875	Muga	RFSp	8	Ll	trans	8.1	26.5	34.0	2.5	dare,kane	loam
71	875	Muga	RFSp	215	Ll	cloudy	7.5	25.7	90.2	2.4	dare,kane	loam
72	600	Muga	RFSp	65	Ll, Larf	trans	7.3	26.0	85.5	2.5	algae,azola,aquatic weed	loam
73	875	Muga	RFSp	6	Ll	trans	7.1	20.5	85.1	0.8	algae,azola,aquatic weed	loam
74	875	Muga	RFSp	8	Ll	trans	6.3	23.5	88.9	0.2	algae,azola,aquatic weed	loam
75	875	Muga	RFSp	7	Ll	trans	7.1	23.8	128.0	2.5	algae,azola,aquatic weed	loam
76	475	Muga	RFSp	251	Ll, Larf	trans	7.9	27.0	122.5	1.6	aquatic weed	sandy
77	500	Muga	RFSp	42	Ll, Larf	trans	7.0	26.2	129.0	1.1	azola	loam
78	525	Muga	RFSp	12	Ll	trans	8.2	36.5	120.0	0.6	algae,azola,aquatic weed	loam
79	375	Muga	RFSt	137	Ll, Larf	trans	8.8	15.1	122.5	3.5	azola,algae	sandy
80	375	Muga	RFSt	0		trans	8.6	18.1	199.6	2.5		sandy
81	350	Muga	River	0		trans	8.2	15.5	120.2	1.3		sandy
82	350	Muga	River	0		trans	8.5	17.5	140.4	2.4		sandy
83	1600	Pakhribas	RFSp	65	Larf	trans	6.8	21.2	60.3	0.3	algae,azola,aquatic weed	clay
84	1600	Pakhribas	RFSp	2	Last	trans	6.6	21.0	87.7	6.5	watercress	clay
85	1700	Pakhribas	RFSp	0		trans	6.7	21.0	32.0	1.1	algae	clay
86	1700	Pakhribas	Spring	0		trans	6.1	18.0	22.0	1.6	aquatic weed,algae	clay
87	1700	Pakhribas	Spring	4	Larf, Last	trans	6.4	22.4	28.0	1.0	aquatic weed,algae	loam
88	1700	Pakhribas	RFSp	0		trans	6.2	22.4	31.8	1.1	algae	loam
89	1600	Pakhribas	RFSt	67	Larf, Last	turbid	6.3	16.3	39.5	2.0	aquatic weed,algae	loam
90	1500	Ghorlikharka	RFSp	3	Larf	trans	7.3	15.0	36.8	0.2	algae,azola,aquatic weed	loam

Type of habitat: RFSp = Rice field fed by spring; RFSt = rice field fed by stream, IC = irrigation chanal; SSt = seepage from stream; PoolSt = pool fed by stream; SSp = seepage from spring.

Snail species: Larf = *L. auricularia* race *rufescens*, Last = *L. auricularia sensu stricto*, Lv = *L. viridis*, Ll = *L. luteola*.

Continued on next page

Appendix Table 4.2.1 (continued)

H No.	Elevation (m)	Location	Type of Habitat	Snail densities (5 man min)	Snail species	Water quality: Colour/ turbidity	pH	Temp (°C)	EC (µs)	DO2 (mg/l)	Vegetation in habitat	Soil type
91	1550	Ghorlikharka	RFSp	4	Larf	trans	7.0	13.0	35.0	1.5	aquatic weed,algae	clay
92	1550	Pakhribas	RFSp	8	Larf	trans	6.5	15.5	83.0	1.8	watercress,aquatic weed	clay
93	1550	Pakhribas	Spring	0		trans	6.6	17.0	57.2	1.7	Eupatorium,ferns	sandy
94	1550	Pakhribas	SSp	6	Larf	turbid	6.0	17.2	43.0	1.5	watercress,Acorus spp,algae,weeds	clay
95	1600	Pakhribas	RFSp	79	Larf	trans	5.8	19.8	72.6	0.5	water cress, azola,algae	clay
96	1650	Pakhribas	Spring	0		trans	6.4	19.5	48.2	0.8	aquatic weed,algae	clay
97	1575	Pakhribas	SSt	9	Larf	trans	6.1	12.6	59.5	0.9	watercress, algae	loam
98	1550	Pakhribas	RFSp	56	Larf, Last	trans	6.5	20.0	55.3	1.2	watercress, algae	loam
99	1550	Pakhribas	Spring	0		trans	5.1	19.1	61.8	0.8	watercress, aquatic weed	loam
100	1550	Pakhribas	RFSp	3	Larf	trans	5.0	17.0	58.5	0.6	algae	loam
101	1600	Pakhribas	RFSp	97	Larf, Last	trans	5.5	18.0	50.7	0.5	water cress	loam
102	1650	Pakhribas	Spring	3	Last	trans	5.8	19.8	37.7	0.9	water cress,algae	sandy
103	1650	Pakhribas	Spring	0		trans	5.1	18.0	42.7	0.3		sandy
104	1750	Pakhribas	Spring	0		trans	5.3	20.0	38.3	0.5	water cress,algae	loam
105	1700	Pakhribas	Spring	0		trans	5.7	22.5	38.3	4.0	water cress,algae	sandy
106	1700	Pakhribas	RFSp	0		turbid	5.4	20.0	30.3	0.5	watercress,Acorus spp,weeds,algae	sandy
107	1750	Pakhribas	RFSp	4	Larf	trans	5.7	14.4	34.8	0.4	watercress,algae	loam
108	1750	Pakhribas	Spring	0		trans	5.2	17.3	49.7	0.3	watercress,algae	sandy
109	1850	Dhankuta	Spring	0		trans	6.1	16.5	60.4	0.5	watercress,algae	sandy
110	1800	Dhankuta	Spring	0		trans	6.0	15.5	63.5	0.3		loam
111	1800	Dhankuta	Spring	0		trans	5.2	18.1	55.1	0.4		loam
112	1550	Dhankuta	RFSp	88	Larf	trans	6.0	20.7	25.2	0.4		loam
113	1750	Dhankuta	Pool	0		trans	5.3	18.0	37.2	0.6		sandy
114	1850	Dhankuta	Spring	0		trans	6.0	18.5	66.3	0.3	watercress,algae	sandy
115	1800	Tankhuwa	Spring	0		trans	6.1	10.3	52.5	0.8	watercress,algae	clay
116	1700	Bhirgaun	Spring	0		cloudy	6.3	14.2	23.5	1.0	algae	loam
117	1750	Bhirgaun	Spring	0		trans	6.8	15.2	26.8	1.1	aquatic weed	loam
118	1770	Bhirgaun	Spring	1	Larf	trans	6.6	14.5	30.3	0.5	algae	loam
119	1700	Bhirgaun	Spring	0		trans	6.1	15.6	23.7	1.0	watercress,algae	sandy
120	1900	Hattikharka	Spring	0		turbid	5.2	10.0	32.2	0.3		sandy
121	1925	Hattikharka	Spring	0		trans	5.9	14.4	32.0	0.2	watercress,aquatic weed	loam
122	1800	Bhirgaun	Spring	0		trans	5.5	17.5	31.0	0.5		sandy
123	1800	Hattikharka	Spring	0		trans	6.2	21.0	17.0	1.5	watercress,algae	loam
124	1800	Tankhuwa	Spring	0		trans	6.2	18.5	18.8	1.3	aquatic weed	loam
125	1950	Tankhuwa	Spring	0		turbid	6.9	19.7	33.7	0.2	watercress,algae	sandy
126	1784	Murtidhunga	Spring	0		trans	6.9	13.6	31.2	0.1	aquatic weed,algae	sandy
127	1700	Parewadin	Spring	0		trans	6.6	11.5	28.8	1.0	aquatic weed,algi,watercress	loam
128	1700	Parewadin	Spring	0		trans	5.1	11.5	26.1	1.0	algae	loam
129	1700	Parewadin	Spring	0		trans	5.8	11.0	31.2	1.4	aquatic weed,algae,watercress	loam
130	1650	Parewadin	Spring	0		trans	6.0	10.0	25.7	1.2	aquatic weed,algae	loam
131	1725	Parewadin	Spring	0		trans	6.6	16.2	61.2	1.2	aquatic weed,algae,watercress	loam
132	1700	Parewadin	Spring	0		trans	6.9	16.1	43.1	1.6	aquatic weed,algae,watercress	loam
133	1600	Parewadin	Spring	0		trans	6.8	16.8	46.7	1.4	aquatic weed,algae,watercress	loam
134	1600	Parewadin	Spring	0		trans	6.1	13.6	33.3	0.6	aquatic weed,algae,watercress	loam
135	1600	Parewadin	Spring	0		trans	6.5	16.3	44.0	1.3	aquatic weed	loam
136	2100	Parewadin	Spring	4	Last	trans	6.2	16.2	26.0	0.9	aquatic weed,algae,watercress	loam
137	1700	Murtidhunga	Spring	0		trans	7.5	13.3	54.4	0.4	aquatic weed,algae,watercress	loam
138	1600	Murtidhunga	Spring	48	Larf, Last	trans	7.2	13.0	32.0	2.3	aquatic weed,algae,watercress	loam
139	1600	Murtidhunga	Spring	0		trans	7.8	8.7	63.0	0.4	weeds,algae,watercress,Acorus spp	loam
140	1650	Murtidhunga	Spring	0		trans	7.5	7.1	32.9	1.6	aquatic weed,algae,watercress	loam
141	2000	Murtidhunga	Spring	0		trans	7.9	13.7	30.0	2.2		loam
142	2000	Murtidhunga	Spring	0		trans	7.7	17.0	27.0	1.8	aquatic weed,algae,watercress	sandy
143	2025	Murtidhunga	Stream	0		trans	6.1	13.5	16.6	3.5		sandy
144	2100	Murtidhunga	Spring	0		trans	5.9	16.5	51.3	1.2	aquatic weed,algae,watercress	clay
145	2180	Murtidhunga	Spring	0		trans	5.4	14.1	24.5	0.7		loam
146	2050	Marekatahare	Spring	0		trans	6.8	18.5	19.7	0.7	aquatic weed,algae,watercress	loam
147	2030	Basantapur	Spring	0		trans	6.5	13.7	38.3	0.3	aquatic weed,algae,Acorus spp	loam
148	2300	Tamaphok	Spring	0		trans	6.1	6.9	85.0	1.5	aquatic weed,algae	loam
149	2250	Tamaphok	Stream	0		trans	6.8	9.8	40.1	1.5	hyacynth	sandy
150	2300	Tamaphok	Spring	0		trans	6.3	12.4	22.7	1.2	aquatic weed,algae	loam
151	2400	Tamaphok	Spring	0		turbid	5.4	12.0	28.3	0.3	aquatic weed,algae	clay
152	2400	Tamaphok	Spring	0		trans	6.3	8.7	27.5	1.0	algae	clay
153	2400	Tamaphok	Spring	0		trans	5.7	11.5	28.7	1.0	aquatic weed,algae	clay
154	2460	Tamaphok	Spring	0		trans	5.2	8.0	17.0	1.3	aquatic weed,algae	clay
155	2460	Tamaphok	Spring	0		trans	5.0	9.4	21.0	1.0	aquatic weed,algae	loam
156	2450	Tamaphok	Spring	0		trans	5.9	6.4	82.6	1.3		clay
157	2600	Tinjure	Spring	0		trans	6.2	6.0	25.7	2.7	aquatic weed	clay
158	2600	Tinjure	Spring	0		trans	6.9	6.4	19.6	0.8	aquatic weed,algae	clay
159	2700	Tinjure	Spring	0		trans	7.7	8.2	26.6	2.1	aquatic weed,algae	clay
160	2800	Tinjure	Pool	0		dark	6.1	6.9	45.8	0.8		clay
161	2850	Tinjure	Pool	0		dark	6.8	8.9	45.8	3.4		clay
162	2700	Phedi	Spring	0		trans	6.9	8.5	14.2	0.8	aquatic weed	clay
163	2700	Phedi	Stream	0		trans	7.3	9.4	17.9	0.8	aquatic weed	loam
164	2600	Chauki	Spring	0		trans	7.9	10.1	13.3	0.2		loam
165	2600	Chauki	Spring	0		trans	6.8	12.4	35.4	0.7	aquatic weed	clay
166	2600	Chauki	Spring	0		turbid	5.7	6.7	16.5	1.1	aquatic weed,algae	clay
167	2400	Chauki	Spring	0		trans	6.6	11.6	20.3	0.9	water cress,aquatic weed,algae	loam
168	3000	Mangalbare	Spring	0		trans	6.6	14.4	23.4	0.3	aquatic weed	clay
169	3000	Mangalbare	Pool	0		dark	6.0	15.5	37.0	0.2		loam
170	3080	Morahang	Spring	0		trans	6.0	15.3	14.5	0.4	water cress,aquatic weed,algae	clay
171	3050	Morahang	Spring	0		trans	6.0	11.9	24.0	0.8	aquatic weed,algae	clay
172	3050	Srimane	Pool	0		turbid	6.1	12.3	57.0	0.9		clay
173	3000	Srimane	Spring	0		turbid	6.6	9.2	13.8	1.8		clay
174	3100	Lampokhari	Pool	0		turbid	5.9	9.0	6.9	0.9	algae	loam
175	3100	Lampokhari	Pool	0		dark	6.6	6.7	8.4	1.9	algae	loam
176	3200	Gupha	Pool	0		dark	6.2	6.1	34.0	1.2		loam
177	3250	Gupha	Pool	0		dark	6.6	6.5	29.0	1.4		loam
178	3200	Gupha	Spring	0		trans	6.2	12.3	25.0	1.1	aquatic weed	clay
179	3200	Dhap	Stream	0		trans	6.3	9.4	39.2	1.6		loam
180	3300	Dhap	Spring	0		trans	6.8	9.2	54.0	1.8		sandy
181	3300	Dhap	Spring	0		trans	6.3	11.9	50.7	1.6	aquatic weed,algae	clay
182	3300	Dhap	Stream	0		trans	6.8	11.4	52.3	1.5		clay
183	3300	Dhap	Pool	0		turbid	6.1	10.5	16.8	1.0	aquatic weed,algae	clay
184	[illegible]	Dhap	Spring	0		trans	6.1	10.4	63.0	0.3	aquatic weed,algae	clay
185	3400	Dhap	Spring	0		trans	7.1	12.1	12.3	0.9	aquatic weed	clay
186	3475	Nundhaki	Spring	0		trans	6.7	9.8	14.1	1.0	aquatic weed	sandy
187	3350	Nundhaki	Spring	0		trans	6.4	9.8	20.2	1.1	aquatic weed	clay

Type of habitat: RFSp = Rice field fed by spring; RFSt = rice field fed by stream, IC = irrigation chanal, SSt = seepage from stream, PoolSt = pool fed by stream, SSp = seepage from spring

Snail species: Larf = *L. auricularia* race *rufescens*, Last = *L. auricularia sensu stricto*, Lv = *L. viridis*, Ll = *L. luteola*

Appendix Table 4.2.2
Cross-sectional Survey
Snail habitats in the Terai (March 1991)

H No.	Elevation (m)	Location	Type of Habitat	Snail densities (5 man min)	Snail species	Water quality					Vegetation in habitat	Soil type
						Colour/ turbidity	pH	Temp (°C)	EC (µs)	DO2 (mg/l)		
188	200	Biratnagar	Pond	0		turbid	5.8	25.6	26.8	4.3	Algae	loam
189	200	Biratnagar	Pond	9	Ll	trans	8.1	27.2	25.5	0.9	Algae	loam
190	200	Biratnagar	Pond	102	Larf, Ll	trans	7.9	27.2	47.3	1.5	Algae,motha,pater	loam
191	200	Biratnagar	Canal	112	Larf, Ll	turbid	8.4	25.7	55.6	1.4	Algae	loam
192	250	Tarahara	RSP	9	Ll	trans	7.5	25.7	46.1	6.7	Azola, algae, aquatic weed	clay
193	250	Tarahara	Canal	0		trans	7.8	24.8	28.0	2.4	Algae	loam
194	250	Tarahara	Canal	0		trans	5.7	27.0	31.0	1.7	Kane	clay
195	250	Santijhoda	RSP	0		turbid	6.7	27.0	46.2	6.2	aquatic weed	clay
196	250	Santijhoda	Canal	0		trans	5.5	20.5	31.9	1.6	aquatic weed	loam
197	250	Santijhoda	Canal	12	Ll, Larf	trans	8.5	20.4	77.1	5.3		clay
198	250	Santijhoda	SR	53	Ll	trans	8.9	20.4	38.2	2.7	Algae, Azola	clay
199	250	Santijhoda	RSP	87	Ll	trans	8.6	27.0	80.6	8.7	motha	clay
200	250	Santijhoda	RSP	8	Ll	turbid	7.3	25.8	45.5	4.7	Algae, Azola,hyacynth,aquatic weed	clay
201	250	Santijhoda	RSP	56	Larf, Ll	trans	7.5	25.4	42.6	2.4	Algae, Azola,llame	clay
202	300	Hansposa	RSP	9	Ll	trans	7.1	22.0	60.9	4.6	Algae, Azola,hyacynth,aquatic weed	loam
203	300	Tarahara	Pond	12	Larf	trans	6.7	20.1	80.9	2.4	Algae, Azola,hyacynth,aquatic weed	loam
204	300	Tarahara	Canal	45	Ll	cloudy	6.9	22.9	57.7	3.4	Aquatic weed	clay
205	300	Tarahara	Canal	0		dark	7.2	20.5	75.3	3.1	Algae, Azola,hyacynth,aquatic weed	loam
206	300	Tarahara	Pond	0		turbid	7.5	25.5	65.2	7.7		clay
207	300	Tarahara	Pond	0		trans	8.4	20.4	28.6	8.7		clay
208	300	Tarahara	Pond	0		turbid	7.8	20.4	31.2	1.7		clay
209	300	Tarahara	SR	0		trans	5.3	27.7	33.3	1.2	Algae, Azola	clay
210	250	Tarahara	Pond	0		turbid	7.8	31.4	24.7	2.7		loam
211	250	Tarahara	RSP	0		turbid	9.6	34.0	69.7	6.4		loam
212	250	Tarahara	RSP	0		turbid	5.4	29.0	55.3	5.2		loam
213	250	Hansposa	RSP	19	Ll	trans	5.4	30.8	38.4	2.7	Algae, hyacinth	clay
214	250	Tarahara	RSP	0		trans	8.2	31.2	51.0	6.3	Algae, hyacinth	loam
215	250	Hansposa	Pond	0		trans	7.8	31.6	46.3	1.1	Algae	loam
216	250	Hansposa	RSP	25	Ll, Larf	trans	7.8	29.5	40.2	3.0	Algae	loam
217	250	Hansposa	RSP	14	Ll	trans	6.8	30.2	52.3	2.4	Algae	clay
218	250	Tarahara	Pond	10	Larf	trans	6.4	32.0	20.6	6.7		clay
219	250	Tarahara	Pond	29	Larf, Ll	cloudy	8.7	20.9	33.4	10.9	Algae, moth	clay
220	250	Tarahara	Canal	2	Ll	trans	9.1	27.1	24.5	6.1	Algae, moth	clay
221	250	Tarahara	Pond	1	Larf	turbid	8.0	26.3	25.0	3.7	Algae, moth	loam
222	150	Bhantabari	RSP	0		cloudy	7.5	25.8	66.3	3.9	Azola, algae	clay
223	150	Bhantabari	Pond	20	Ll, Larf	trans	7.8	27.0	55.2	5.1	Azola, algae	loam
224	150	Bhantabari	RSP	1	Ll	turbid	7.8	29.0	67.8	4.5	Algae, hyacinth	loam
225	150	Bhantabari	Pond	4	Ll	trans	7.8	28.2	54.5	4.6		loam
226	150	Bhantabari	SC	17	Ll	turbid	7.3	28.4	35.7	6.1	aquatic plants	loam
227	150	Bhantabari	SR	55	Larf	trans	7.2	25.2	120.8	6.6	Algae	sandy
228	150	Bhantabari	Pond	15	Larf, Ll	trans	7.3	30.8	113.6	4.3	Azola, algae	loam
229	150	Bhantabari	River	1	Ll	trans	6.9	31.3	139.9	6.2	aquatic plants	clay
230	150	Bhantabari	River	0		trans	7.3	29.8	144.8	1.5	aquatic plants	sandy
231	150	Bhantabari	RSP	0		turbid	6.6	27.2	197.0	5.8	aquatic plants	clay
232	200	Jhumka	RSP	38	Larf, Ll	turbid	7.5	33.9	182.3	2.9		clay
233	200	Jhumka	RSP	4	Larf	turbid	7.1	37.3	174.8	9.0		clay
234	200	Jhumka	RSP	0		turbid	7.2	31.2	163.3	4.9		clay
235	200	Jhumka	Pond	0		trans	7.5	33.6	137.5	6.8		clay
236	200	Jhumka	Pond	0		trans	7.6	33.2	168.5	5.8		loam
237	200	Jhumka	Pond	0		trans	5.8	35.1	107.9	7.9		sandy
238	200	Jhumka	Canal	0		trans	5.4	33.5	196.8	9.8		clay
239	200	Jhumka	Pond	9	Larf	cloudy	8.1	33.8	168.3	7.6	Algae, aquatic plants	clay
240	200	Jhumka	Pond	0		cloudy	7.8	32.8	142.9	6.4		loam
241	200	Jhumka	Pond	69	Larf, Ll	trans	7.3	33.3	156.3	2.1	Algae, aquatic plants	loam
242	200	Jhumka	Pond	109	Larf, Ll	trans	7.3	31.4	164.8	5.0	Algae, aquatic plants	loam
243	225	Jhumka	Canal	0		cloudy	8.4	32.7	178.8	6.4		sandy
244	225	Jhumka	Canal	0		turbid	7.5	32.8	177.4	4.1	Algae, aquatic plants	loam
245	225	Jhumka	Pond	2	Larf	turbid	7.8	20.5	166.0	4.7	Algae, aquatic plants	loam
246	225	Jhumka	RSP	3	Larf	trans	8.4	29.5	178.1	10.9		loam
247	225	Jhumka	RSP	0		turbid	7.1	29.4	175.2	7.1	Azola	clay
248	225	Jhumka	RSP	15	Larf	turbid	8.3	28.4	131.6	9.3	Kane, algae	clay
249	325	Letang	Canal	0		trans	8.4	28.3	208.0	4.4	Pire jhar(a fish poison)	sandy
250	325	Letang	Pond	0		turbid	10.6	29.8	218.0	3.2	Algae	sandy
251	325	Letang	DDW	830	Ll, Larf	turbid	9.3	23.6	209.0	1.1	Aquatic weed	loam
252	300	Letang	DDW	500	Ll, Larf	trans	8.7	23.8	212.0	3.8		cement
253	300	Letang	RSP	0		turbid	7.9	24.5	240.0	9.5		loam
254	300	Letang	Canal	0		trans	9.4	23.0	205.0	6.9		sandy
255	300	Letang	RSP	0		trans	8.6	24.5	206.0	3.5		loam
256	300	Letang	DDW	123	Ll	cloudy	7.5	26.5	240.0	5.4	Algae	loam

Type of habitat: RSP = road side pool; SR = seepage from river; SC = seepage from canal; DDW = ditches around drinking water place.

Snail species: Larf = *L. auricularia* race *rufescens*; Ll = *L. luteola*.

Appendix Table 4.2.3

Longitudinal Survey

Changes in physical and chemical properties of water and snail densities in habitats in the hills

MONTH		Habitat No	1	2	3	4	5	6	7	8	9	10	11	12	13
		Habitat type	RFSt	RFSt	RFSp	RFSp	RFSp	RFSp	RFSp	RFSt	RFSp	RFSp	RFSp	RFSp	RFSp
		Altitude	400	475	875	875	1100	1100	1300	1150	1150	1100	1500	1400	1450
APRIL (1991)	Physical and chemical properties	pH	7.7	6.3	6.5	7.9	6.1	7.2	6.5	7.3	7.1	5.3	8	7.1	7.1
		Temparature	19.1	24.6	24.5	26.7	21.2	19.5	19.2	20	22.7	21.1	21	16	14.5
		D. Oxygen	1.2	6.3	3	1.1	2.9	4.5	1.3	6.3	3.3	0.3	1.3	3.9	2.2
		EC	108.2	82	78	77.3	58	43	66.5	68	45	2.4	18.6	52.2	24.4
		Habitat size	2000	800	2500	1600	800	200	200	100	50	100	2000	100	100
	Snail population (5 min count)	Egg masses	++	+	+	++	+	-	-	+	-	-	-	-	-
		< 5mm	344	0	0	466	85	0	0	0	0	0	0	0	0
		> 5 mm	406	600	1	284	365	0	1000	750	0	12	31	18	70
		Total	750	600	1	750	450	0	1000	750	0	12	31	18	70
		Dead snails	0	0	1	2	10	0	30	0	0	0	0	0	0
MAY (1991)	Physical and chemical properties	pH	7.5	7.6	7.6	8.2	6.9	7.5	7.3	7.2	6.5	6.1	6.6	6.8	6.8
		Temparature	30.2	27.4	30	33.4	21.6	22.5	18.8	22.1	22	20	21	18.5	18.1
		D. Oxygen	1.5	3.6	0.2	2.2	2.8	2.5	3.2	4.7	2	0.5	0.6	1.3	1.3
		EC	92	81.8	59.6	51.6	60.7	55	49.5	52.1	39	60	38	75	81.4
		Habitat size	2000	1200	3000	1600	800	200	150	150	50	200	2500	200	150
	Snail population (5 min count)	Egg masses	++	+++	++	-	++	++	+	+	-	-	-	-	+
		< 5mm	0	4	0	4	0	0	0	0	0	0	0	0	0
		> 5 mm	63	196	36	146	334	625	135	300	0	70	30	170	50
		Total	63	200	36	150	334	625	135	300	0	70	30	170	50
		Dead snails	25	200	60	6	3	0	4	0	0	6	3	0	1
JUNE (1991)	Physical and chemical properties	pH	7.3	7.1	6.8	6.7	6.8	7	7	7.1	6.9	7.2	6.6	6.8	6.8
		Temparature	26	26	26	25	22	23	25	26	21	24	20	22	20
		D. Oxygen	4.5	2.1	0.7	2.1	0.6	2.2	1.5	2.1	1.4	1.3	3.1	2.2	0.8
		EC	74.2	73.5	48.3	63.2	57.3	33.3	25.5	37.1	35.2	61	31.8	35.3	51.5
		Habitat size	2000	1500	4000	5000	1500	1500	500	1000	100	400	3000	1000	500
	Snail population (5 min count)	Egg masses	+	+	-	++	+	-	+	+	-	+	-	-	-
		< 5mm	5	140	44	0	27	20	0	6	0	0	0	0	0
		> 5 mm	8	240	116	37	133	125	32	21	0	19	0	46	380
		Total	13	380	160	37	160	145	32	27	0	19	0	46	380
		Dead snails	6	13	5	4	5	1	1	1	0	1	1	0	0
JULY (1991)	Physical and chemical properties	pH	7.3	7.3	6.5	7.2	7.2	7.2	7.4	7.1	7.1	6.9	6.8	7.2	7
		Temparature	35	32	30	34	22	24	27	23	21	22	21	22	22
		D. Oxygen	6.1	0.6	1.9	3.7	0.8	5.6	0.4	4.4	4.3	2.1	0.9	1	0.8
		EC	127	37.8	50	33.4	57.1	44.1	54.7	56.7	41.5	60	34.5	37	22
		Habitat size	2000	1500	4000	6000	2000	1500	600	1000	100	500	3000	1500	800
	Snail population (5 min count)	Egg masses	+	-	-	++	-	-	++	++	-	+	-	-	+
		< 5mm	3	0	0	40	13	0	0	9	0	0	0	0	0
		> 5 mm	6	150	6	9	9	10	355	5	0	30	2	80	20
		Total	9	150	6	49	22	10	355	14	0	30	2	80	20
		Dead snails	0	110	6	1	5	0	10	0	0	0	0	0	0

MONTH		Habitat No	14	15	16	17	18	19	20	21	22	23	24	25	26
		Habitat type	RFSp	RFSp	RFSp	RFSp	Spng	Spng	RFSp	Spng	Spng	Spng	Spng	Spng	Spng
		Altitude	1400	1550	1500	1600	1550	1600	1750	1850	1850	1975	1975	2100	1925
APRIL (1991)	Physical and chemical properties	pH	7.5	7.1	6.7	6.3	6.4	7.3	7.4	7.7	6.4	6.7	6.3	5.9	6.1
		Temparature	18	14.5	13.2	25.6	14.4	15	22.2	17.3	18.4	22.4	12.5	17.3	26.2
		D. Oxygen	4.6	2.2	2.3	4.7	0.5	0.8	1	0.3	0.6	0.1	-2.5	-1.7	2.1
		EC	36	24.4	53	42.7	39	22	40.1	54.4	49.2	29.5	21.6	22.2	45.7
		Habitat size	400	30	250	1000	100	50	30	50	50	25	50	500	2000
	Snail population (5 min count)	Egg masses	-	-	-	-	-	-	-	-	-	-	-	-	+
		< 5mm	0	1	0	0	0	0	0	0	0	0	0	0	9
		> 5 mm	9	10	15	160	28	0	430	0	0	0	0	0	16
		Total	9	11	15	160	28	0	430	0	0	0	0	0	25
		Dead snails	3	35	3	1	0	0	3	0	0	0	0	0	0
MAY (1991)	Physical and chemical properties	pH	7.9	6.2	5.5	6.3	6.4	5.9	6	6.6	6.9	6.5	6.3	7.7	7.4
		Temparature	19	23.2	24.5	20	22.3	18.6	22	20	13	16	14	16.5	15
		D. Oxygen	0.2	0.2	2.3	0.2	1.6	0.1	1.6	0.2	0.1	-0.2	-2.7	-1.5	2
		EC	28	54.2	72.2	43	33	31.2	47.8	44.7	44.7	25.4	23	31.2	35.2
		Habitat size	500	50	300	1500	150	50	30	50	50	25	100	1000	2500
	Snail population (5 min count)	Egg masses	+	-	+	+	-	-	+	-	-	-	-	-	+
		< 5mm	0	0	0	0	0	0	0	0	0	0	0	0	7
		> 5 mm	33	0	245	60	85	0	130	0	0	0	0	0	23
		Total	33	0	245	60	85	0	130	0	0	0	0	0	30
		Dead snails	1	37	0	6	0	0	2	0	0	0	0	0	0
JUNE (1991)	Physical and chemical properties	pH	6.8	6.7	6.9	6.6	6.8	6.7	6.1	6.6	6.7	6.7	6.6	6.6	6.8
		Temparature	19	23	24	20	19	18	21	20	20	20	17	17	21
		D. Oxygen	0.3	0.7	2	1.7	0.8	0.3	0.4	0.1	0.4	-0.8	-3.8	-5.8	0.3
		EC	33.1	39.8	71.7	57.7	40.3	58.3	44.8	72.4	72.4	54.4	51.3	57.4	48.6
		Habitat size	600	100	400	2000	300	80	200	100	50	50	100	1000	3000
	Snail population (5 min count)	Egg masses	-	-	-	-	-	-	-	-	-	-	-	-	-
		< 5mm	0	0	0	0	0	0	0	0	0	0	0	0	4
		> 5 mm	360	0	125	170	0	0	175	0	0	0	0	0	20
		Total	360	0	125	170	0	0	175	0	0	0	0	0	24
		Dead snails	1	1	0	3	0	0	0	0	0	0	0	0	1
JULY (1991)	Physical and chemical properties	pH	6.9	6.8	6.8	6.8	6.9	6.9	7.1	7	6.7	7.4	7.3	7.1	7.1
		Temparature	20	23	24	23	19	18	21	20	19	18	18	18	28
		D. Oxygen	0.8	0.5	1.6	0.6	0.6	0.2	0.8	0.1	0.2	-0.5	-6.7	-0.8	0.9
		EC	34.2	41	55.2	51	36.2	30.7	22.5	38	40.6	21.5	24.2	28	39.5
		Habitat size	700	200	400	3000	400	100	200	100	50	50	100	1000	3500
	Snail population (5 min count)	Egg masses	+	-	-	+	-	-	++	-	-	-	-	-	-
		< 5mm	0	0	0	0	0	0	0	0	0	0	0	0	0
		> 5 mm	120	12	7	90	0	0	50	0	0	0	0	0	14
		Total	120	12	7	90	0	0	50	0	0	0	0	0	14
		Dead snails	0	0	1	0	0	0	1	0	0	0	0	0	1

Habitat type: RFSp = ricefield fed by spring; RFSt = ricefield fed by stream; Spng = spring.

Physical and chemical properties of water: Temperature of water in °C; D. oxygen = dissolved oxygen in mg/l; EC = electro-conductivity of water in µs; Habitat size in square metre.

Presence of egg masses: +++ = abundant; ++ = moderate; + = scanty; - = absent.

** = habitat dried up

Continued on next page

Appendix Table 4.2.3 (continued)

MONTH		Habitat No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
		Habitat type	RFSt	RFSt	RFSp	RFSp	RFSp	RFSp	RFSp	RFSt	RFSp	RFSp	RFSp	RFSp	RFSp	RFSp	RFSp	RFSp	RFSp	Spng	Spng	RFSp	Spng	Spng	Spng	Spng	Spng	Spng
		Altitude	400	475	875	875	1100	1100	1300	1150	1150	1100	1500	1400	1450	1400	1550	1500	1600	1550	1600	1750	1850	1850	1975	1975	2100	1925
AUGUST (1991)	Physical and chemical properties	pH	7.1	7.3	7.1	6.9	7	7.2	6.9	7.1	7.1	7	6.9	7	7	7	7.2	7	7	7.1	7.1	7.1	6.8	7	7.1	7.1	7.2	7.1
		Temparature	30	35	32	27	22	24	21	24	21	26	20	25	23	20	26	23	23	18	17	27	19	19	16	18	17	23
		D. Oxygen	3.4	3.8	3.7	2.8	1.5	5	2.4	3.4	3.3	1.9	2.3	2.7	3.3	2.3	1.1	1.1	0.6	1.2	0.6	1.1	1	1	0.9	0.9	0.8	0.9
		EC	94.1	37.8	94.2	37.3	35	52	88.2	68.2	65.5	90	54.2	62	58.8	32.2	34.2	54.2	51.4	64.2	67.2	84.2	41.3	28.2	51	72.2	46.8	42.3
		Habitat size	2000	1500	3500	6000	2200	1500	1000	1000	100	500	3000	1500	800	700	200	300	2500	400	100	150	100	50	50	80	1000	3500
	Snail population (5 min count)	Egg masses	-	-	-	-	+	++	-	++	-	+	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-
		< 5mm	2	0	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
		> 5 mm	92	0	2	0	0	12	71	16	0	29	1	0	37	17	1	1	33	0	0	35	0	0	0	0	0	0
		Total	94	0	2	4	0	12	71	17	0	29	1	0	37	17	1	1	33	0	0	37	0	0	0	0	0	0
		Dead snails	4	0	1	0	0	7	15	0	0	1	0	0	1	1	0	0	2	0	0	2	0	0	0	0	0	1
SEPTEMBER (1991)	Physical and chemical properties	pH	6.8	7	7.1	6.9	6.7	7	6.6	7	6.9	6.9	6.8	6.9	7	6.8	7.1	7	7.1	7	6.9	7.1	6.8	7	7.1	6.9	7	7
		Temparature	28	31	32	25	21	24	21	21	20	24	20	21	21.5	19	25	21	22	18	17	18	19	20	17	18	18	20
		D. Oxygen	3.2	3.2	2.8	3.3	1.3	3.9	1.4	2.1	3	1.9	1.6	1.7	1.2	2.6	1.5	1.5	1	1	0.5	1	0.2	0.1	0.4	1	0.7	1
		EC	84.2	78.4	84.4	50	96.4	109	60.3	64	96.2	86.2	54.4	67.2	83.4	64.8	91.2	53.2	38.5	26.5	65.2	81.2	73	63.2	83.2	61.2	38.2	50
		Habitat size	2000	1500	2000	5000	2000	1500	800	1000	100	500	3000	1000	800	600	100	300	2500	350	80	100	50	50	50	50	800	3000
	Snail population (5 min count)	Egg masses	-	-	-	-	+	++	-	++	-	+	+	+	++	++	-	+	-	-	-	+	-	-	-	-	-	-
		< 5mm	6	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		> 5 mm	21	0	0	0	12	1	59	21	0	37	27	1	41	29	0	27	41	0	0	19	0	0	0	0	0	0
		Total	27	0	0	0	13	3	59	21	0	37	27	1	41	29	0	27	41	0	0	19	0	0	0	0	0	0
		Dead snails	2	0	0	0	0	0	2	3	0	2	0	0	3	2	0	0	1	0	0	1	0	0	0	0	0	0
OCTOBER (1991)	Physical and chemical properties	pH	6.9	7.9	8.4	7.9	8.6	7.13	6.9	7.8	7.5	7.6	7.1	6.6	7.1	7.4	6.5	6.1	5.9	7.8	7.8	7.3	7.3	7.3	7.2	7.2	7.6	7.3
		Temparature	27	38.5	28	28.4	28.8	21.4	28.8	19.6	19.4	26.6	19.6	21.1	21	22.2	21.9	21.7	24.6	16.6	16.4	16.1	18.4	19.4	19.2	20.1	19.4	21.7
		D. Oxygen	1.1	2.2	8.2	2.7	0.5	2.6	2.1	1.2	5.5	1.5	4.1	2.9	5.1	5.5	2.5	2.3	1.9	3.1	2.4	1.2	3.2	4.4	1.5	0.9	2.5	4.8
		EC	71.6	39.9	37.1	66.8	21.4	36.6	68.8	32.6	21.2	53.2	51.2	64.3	28.9	42.4	15.7	49.1	22.2	30.1	30.7	29.4	32.7	49.3	23.3	38.7	20.1	33.3
		Habitat size	2000	1200	2000	3000	1800	1000	700	800	100	400	3000	800	600	500	50	250	2500	300	80	80	50	50	25	50	700	3000
	Snail population (5 min count)	Egg masses	-	-	-	++	-	+	-	++	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-
		< 5mm	0	0	0	8	0	1	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
		> 5 mm	0	110	0	142	10	29	40	13	0	25	50	35	75	50	0	15	0	0	0	20	0	0	0	0	0	13
		Total	0	110	0	150	10	30	40	20	0	25	50	35	75	50	0	15	0	0	0	20	0	0	0	0	0	18
		Dead snails	0	0	0	0	0	0	2	8	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0
NOVEMBER (1991)	Physical and chemical properties	pH	7.3	7.4	7.3	7.3	6.8	6.9	7	7	6.9	6.7	6.9	7	7	6.9	7.2	7.9	7.3	7.2	7	7.1	7.1	7.4	6.9	7.2	7	7
		Temparature	23	22	23	24	21	17.5	14.5	20	19	23	17	10.1	14	16.5	17	18	17	14	13.2	15	15	14.4	15	14	16.2	17.2
		D. Oxygen	2.2	3	2.7	1.3	1.3	2.3	2.3	3.3	1.4	1.1	2.7	1.8	1.6	3.4	2.1	2.4	1.7	3	1	1.1	1.1	3	3.4	0.7	3.6	3.8
		EC	150	80.2	87.6	81.2	70.6	61.5	66.8	51.7	39.4	62.4	33.4	66.5	45.6	35	5.1	52.4	40	41.2	56.2	18	41.4	61.3	31.3	55.2	40.4	44.2
		Habitat size	1500	1000	2000	2500	1500	500	250	500	80	300	2000	500	200	500	50	250	2000	300	50	50	50	50	25	50	600	2500
	Snail population (5 min count)	Egg masses	-	-	-	-	++	-	+	++	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		< 5mm	0	0	0	0	0	1	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		> 5 mm	0	0	0	1	20	1	33	13	0	0	65	71	58	1	0	16	0	0	0	15	0	0	0	0	0	0
		Total	0	0	0	1	20	2	33	18	0	0	65	71	58	1	0	16	0	0	0	15	0	0	0	0	0	0
		Dead snails	0	0	0	0	0	0	1	0	0	0	13	10	2	0	0	3	0	0	0	2	0	0	0	0	0	0
DECEMBER (1991)	Physical and chemical properties	pH	7.1	7.3	7.2	7.2	7	7	7.1	7.3	7.3	7.3	7.3	7.1	7.1	7.2	7.3	7.2	7.1	7.1	7.2	7	7	7.2	7	7	6.9	7.1
		Temparature	17	24	22	24	20	19	21	17	17	17	15	10	13	15	17	19	19	15	16	14	14	13	14.5	13	14	14
		D. Oxygen	3.1	1.4	3.7	2.7	1.2	4	0.8	2.1	44	3	1.9	2	2.3	2.7	1.9	1	2.3	3.7	1.2	1.3	0.3	2.5	1.9	2.1	0.4	0.9
		EC	87.2	66.8	58.8	91.3	74.3	71.2	69.9	63.1	35.8	62.4	32.4	59.2	48	37.2	34.3	42.6	50.6	54.5	30.9	34.8	36.2	58.2	41.2	30.3	51.4	32.2
		Habitat size	1000	800	1500	2000	1500	300	200	200	50	200	2000	300	200	500	50	250	1500	200	50	50	50	50	25	50	500	2500
	Snail population (5 min count)	Egg masses	+++	+	+++	-	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-
		< 5mm	0	22	0	0	11	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		> 5 mm	64	62	17	0	15	0	26	8	0	20	71	41	33	0	0	1	35	0	0	15	0	0	0	0	0	0
		Total	64	84	17	0	26	0	26	20	0	20	71	41	33	0	0	1	35	0	0	15	0	0	0	0	0	0
		Dead snails	3	6	0	0	0	0	0	10	0	0	3	2	1	0	0	0	2	0	0	0	0	0	0	0	0	0

Habitat type: RFSp = ricefield fed by spring; RFSt = ricefield fed by stream; Spng = spring.

Physical and chemical properties of water: Temperature of water in °C; D. oxygen = dissolved oxygen in mg/l; EC = electro-conductivity of water in µs; Habitat size in square metre.

Presence of egg masses: +++ = abundant; ++ = moderate; + = scanty; - = absent.

** = habitats dried up.

Continued on next page

Appendix Table 4.2.3 (continued)

MONTH		Habitat No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
		Habitat type	RFSt	RFSt	RFSp	RFSp	RFSp	RFSp	RFSp	RFSt	RFSp	RFSp	RFSp	RFSp	RFSp	RFSp	RFSp	RFSp	RFSp	Spng	Spng	RFSp	Spng	Spng	Spng	Spng	Spng	Spng
		Altitude	400	475	875	875	1100	1100	1300	1150	1150	1100	1500	1400	1450	1400	1550	1500	1600	1550	1600	1750	1850	1850	1975	1975	2100	1925
JANUARY (1992)	Physical and chemical properties	pH	7.2	7.1	7.1	7.9	7.2	7.1	7.2	**	7.3	7.3	6.1	6.3	6.2	7	**	6.4	7.4	6.4	6.4	7.3	7.4	6.8	6.7	6.2	7.1	7.2
		Temparature	15	25	20	23	18	22	16	**	14	17	21	11	13.2	13	**	18.1	19.3	12	15	14	15	18	12	14	12	12
		D. Oxygen	1.5	2	2.2	3	0.9	2.6	3.1	**	1	2.1	0.3	3	1.3	1.8	**	1.5	0.8	3.6	2.1	0.4	2.6	2.9	0.4	1.6	0.9	1.6
		EC	130	97	54	79	80	80	85.3	**	44.4	54.2	38.9	44.9	37	74.9	**	47.7	48.2	46	50	42.1	50	61.2	53.2	24.8	29.5	22.2
		Habitat size	500	600	1200	1600	1200	200	200	**	50	200	1500	300	200	500	**	250	1000	100	50	50	50	50	25	50	500	2500
	Snail population (5 min count)	Egg masses	+++	+++	++	+	++	++	++	**	-	-	-	+	+	-	**	-	++	-	-	+	-	-	-	-	-	++
		< 5mm	217	3	326	0	8	10	0	**	0	0	0	0	0	0	**	0	0	0	0	0	0	0	0	0	0	2
		> 5 mm	198	26	144	17	28	126	135	**	0	1	21	95	35	0	**	0	46	0	0	57	0	0	0	0	0	25
		Total	415	29	470	17	36	136	135	**	0	1	21	95	35	0	**	0	46	0	0	57	0	0	0	0	0	27
		Dead snails	100	9	1	6	0	0	0	**	0	0	0	0	15	1	**	2	13	0	0	17	0	0	0	0	0	4
FEBRUARY (1992)	Physical and chemical properties	pH	7.1	6.4	7.5	7.1	6.5	6.8	6.7	7	6.4	6.3	6.5	7.2	6.4	7.4	**	6.4	8	6.9	6.1	6.4	7.5	6.4	6.8	6.4	7.2	7.4
		Temparature	18	18	14	18	13	20	14	12	15	18.1	14	12	12	13	**	19	13	16	15	13	16.3	19	11	12	14	12
		D. Oxygen	2.3	2.1	1	1	1	1	2.1	1	2	4	1.1	2.9	2.1	1.8	**	2	1.3	2.4	1.2	0.2	1.2	1.9	0.3	0.8	1	1.7
		EC	96.5	89	88.5	81.2	63	83.1	60.1	65	49.6	61	33.9	44.8	63	35.9	**	48.7	50.6	48	49.4	41.1	51	58.4	57.1	50	31.9	44.1
		Habitat size	400	600	1200	1500	1000	100	100	100	50	200	2000	250	200	500	**	250	1000	100	50	50	50	50	25	50	500	2000
	Snail population (5 min count)	Egg masses	++	++	-	+	+	++	+	++	-	+	+	++	++	-	**	-	+	-	-	-	-	-	-	-	-	-
		< 5mm	29	90	15	0	14	0	0	0	0	0	0	0	0	0	**	0	9	0	0	0	0	0	0	0	0	5
		> 5 mm	74	180	10	225	86	110	110	13	0	7	21	98	275	0	**	0	37	0	0	20	0	0	0	0	0	12
		Total	103	270	25	225	100	110	110	13	0	7	21	98	275	0	**	0	46	0	0	20	0	0	0	0	0	17
		Dead snails	12	19	5	7	0	1	0	10	0	1	9	15	28	0	**	4	13	0	0	3	0	0	0	0	0	2
MARCH (1992)	Physical and chemical properties	pH	7.4	7.3	7.3	7.3	7.2	7.3	7.3	7.3	7.3	7.4	7.3	7.3	7.4	7.3	**	7.2	7.2	7	7.1	7.3	7.3	7.3	7.2	7.2	7.2	7.3
		Temparature	24	24	27	28	21	20	22	17	17	18	13	13	12	14	**	17	15	15	15	14	17	19	18	14	14	14
		D. Oxygen	3.2	2.2	1.4	0.9	0.5	2.5	1.2	2.2	2.1	2.9	1.3	2.8	2.1	2	**	1.8	1.9	3	1	1.1	1	1.7	0.2	2	0.5	2.4
		EC	98.4	86	78.2	85.2	73.6	63	56.8	73.3	44.3	50.8	36.4	46.6	79.3	49.3	**	53.6	60.3	72	36.8	42.4	48	50.7	28.7	35.8	26.7	33.7
		Habitat size	400	600	1200	1500	800	250	100	100	50	100	2000	250	200	500	**	250	1000	100	50	50	50	50	25	50	500	2000
	Snail population (5 min count)	Egg masses	+++	+++	+	+	+	++	-	+++	-	+	-	-	+	-	**	+	-	-	-	+	-	-	-	-	-	+
		< 5mm	510	31	0	30	0	43	0	163	0	0	0	0	0	0	**	2	0	0	0	0	0	0	0	0	0	0
		> 5 mm	90	89	25	100	188	557	0	163	0	70	28	375	200	0	**	19	57	0	0	26	0	0	0	0	0	20
		Total	600	120	25	130	188	600	0	325	0	70	28	375	200	0	**	21	57	0	0	26	0	0	0	0	0	20
		Dead snails	0	0	0	0	0	0	0	0	0	1	37	4	0	0	**	0	6	0	0	2	0	0	0	0	0	2
APRIL (1992)	Physical and chemical properties	pH	7.5	7.4	6.7	7.4	6.5	6.5	6.1	6.3	7.3	6.7	7.3	6.3	6.4	7.5	**	7.5	6.7	7	6.5	6.3	7.3	7.2	6.2	6.1	7.2	6.2
		Temparature	23	23	34	23	18	20	23	18	20	21	13	21	13	17	**	16	20	16	25	22	23	22	14.2	12	16	12
		D. Oxygen	3.7	1.9	3	1.9	1.4	2	2	3.1	2.1	2	0.3	0.8	1	1.1	**	1.9	0.2	3.2	0.2	1.7	2.4	1.3	0.2	1.2	2	1.1
		EC	133	84.3	68.5	84.2	69.2	54.2	108.2	96.4	73.1	38.6	36.4	61.3	51.3	57.4	**	83.4	61.9	76	51.3	57.3	53.2	49.6	37.7	61	39.9	46.1
		Habitat size	500	1000	2000	2000	1000	250	250	100	50	200	2000	250	200	500	**	250	1000	100	50	50	50	50	25	50	500	2000
	Snail population (5 min count)	Egg masses	+++	+++	+	+	++	++	+++	+	-	+	-	+	++	-	**	+	+	+	-	+	-	-	-	-	-	+
		< 5mm	101	268	0	13	0	106	0	29	0	0	0	0	0	0	**	3	0	0	0	0	0	0	0	0	0	0
		> 5 mm	499	432	29	71	700	244	1005	391	0	51	0	17	107	0	**	21	40	11	0	25	0	0	0	0	0	12
		Total	600	700	29	84	700	350	1005	420	0	51	0	17	107	0	**	24	40	11	0	25	0	0	0	0	0	12
		Dead snails	10	0	5	1	0	2	15	10	0	5	0	2	5	0	**	3	0	0	0	0	0	0	0	0	0	0

Habitat type: RFSp = ricefield fed by spring; RFSt = ricefield fed by stream; Spng = spring.

Physical and chemical properties of water: Temperature of water in °C; D. oxygen = dissolved oxygen in mg/l; EC = electro-conductivity of water in µs; Habitat size in square metre.

Presence of egg masses: +++ = abundant; ++ = moderate; + = scanty; - = absent.

** = habitats dried up.

Appendix Table 4.2.4
Longitudinal Survey
Changes in physical and chemical properties of water and snail densities in habitats in the Terai

Month	Habitat No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	Habitat type	SC	DDW	RSP	ICH	RSP	RSP	RSP	RSP	RVR	RSP	RSP	DDW	ICL	RVR	DDW	ICH	RSP	RVR
	Location	Tar	Tar	Tar	Tar	Tar	Bha	Bha	Bha	Bha	Jhu	Jhu	Jhu	Jhu	Jhu	Let	Let	Let	Let
APR	pH	9.2	7.7	9.8	7.2	8.5	7.8	7.2	9.0	8.2	7.5	8.8	7.3	7.8	8.9	9.3	7.5	8.0	7.7
	Temparature	34.9	29.6	31.9	31.8	30.3	27.0	33.7	28.7	32.0	33.9	37.4	31.4	20.5	28.5	30.7	26.9	30.3	33.6
(1991)	D. Oxygen	13.5	19.4	9.7	1.3	4.9	5.1	24.4	10.7	9.6	2.9	7.8	5.0	4.7	9.3	21.5	16.9	8.3	17.1
	EC	133.8	134.4	135.9	57.7	135.6	55.2	66.5	119.6	178.2	182.3	147.5	164.8	166.0	149.7	198.2	259.0	260.0	240.0
	Egg masses	+++	-	+++	+++	+++	-	+++	++	-	-	++	-	-	-	+++	+++	+++	-
	< 5mm	73	0	194	0	482	0	20	4	0	0	32	0	0	0	89	108	54	0
	> 5 mm	653	0	681	225	293	0	250	16	0	0	18	0	0	0	221	542	396	0
	Total	726	0	875	225	775	0	270	20	0	0	50	0	0	0	310	650	450	0
	Dead snails	0	0	0	37	0	0	25	0	0	0	25	0	0	0	12	0	0	0
MAY	pH	8.6	8.1	8.5	**	8.6	7.4	7.4	7.4	7.2	**	8.0	7.4	8.1	8.8	8.2	7.9	7.7	7.5
	Temparature	27.2	36.4	31.8	**	31.6	31.6	32.9	29.7	29.2	**	27.0	27.2	28.1	27.8	24.9	25.4	28.3	32.3
(1991)	D. Oxygen	14.7	15.2	13.0	**	15.5	2.7	9.9	33.7	22.3	**	13.5	3.8	34.9	8.9	16.5	1.8	4.5	15.5
	EC	131.1	109.1	161.1	**	142.2	31.2	142.5	78.1	140.1	**	134.5	136.3	91.4	163.2	188.5	173.8	187.5	191.7
	Egg masses	+++	+++	+++	**	+++	-	+++	-	-	**	+++	-	-	-	++	-	+++	-
	< 5mm	17	0	0	**	7	0	30	0	0	**	12	0	0	0	83	0	134	0
	> 5 mm	153	325	530	**	218	0	560	0	0	**	225	0	0	0	83	0	183	0
	Total	170	325	530	**	225	0	590	0	0	**	237	0	0	0	165	0	317	0
	Dead snails	60	5	11	**	51	0	0	0	0	**	12	0	0	0	51	0	25	0
JUN	pH	**	7.7	7.1	7.4	*	7.2	7.6	7.4	8.1	7.6	7.3	8.3	7.8	7.6	7.9	7.4	7.2	7.5
	Temparature	**	30.7	31.2	32.7	*	34.2	35.1	32.1	28.9	32.9	34.6	30.8	32.9	31.5	33.7	30.3	34.6	33.0
(1991)	D. Oxygen	**	8.8	14.9	8.5	*	10.6	13.0	6.3	8.1	3.9	9.5	1.1	5.1	4.8	1.0	4.3	11.1	14.1
	EC	**	85.1	66.6	65.1	*	35.4	76.2	68.5	92.2	61.9	85.8	97.2	75.1	63.3	55.7	124.3	88.7	69.5
	Egg masses	**	++	-	+	*	+	-	-	-	+	+	-	-	-	+	+	+++	-
	< 5mm	**	5	0	14	*	0	0	0	0	0	0	0	0	0	7	5	138	0
	> 5 mm	**	120	0	14	*	3	0	0	0	5	11	0	0	0	5	23	113	0
	Total	**	125	0	27	*	3	0	0	0	5	11	0	0	0	12	28	250	0
	Dead snails	**	11	0	3	*	0	0	0	0	0	6	0	0	0	7	10	110	0
JUL	pH	8.3	7.7	7.9	7.9	*	8.1	7.1	8.2	8.6	10.7	7.1	7.4	7.7	7.4	7.6	7.8	8.6	7.2
	Temparature	33.3	27.9	32.7	36.6	*	32.6	31.2	35.3	31.6	38.3	40.7	35.1	35.1	37.6	28.5	24.3	33.5	39.2
(1991)	D. Oxygen	1.3	1.1	4.8	1.6	*	7.8	5.3	2.2	1.7	2.4	3.8	5.0	5.4	3.7	3.4	1.8	1.4	7.0
	EC	148.3	33.0	145.0	138.7	*	146.5	166.6	74.6	158.8	67.4	64.4	165.5	90.0	145.3	80.6	120.3	123.9	108.8
	Egg masses	-	-	++	-	*	-	+++	-	-	-	-	+	-	-	-	-	+++	-
	< 5mm	0	0	0	0	*	0	0	0	0	0	0	0	0	0	0	0	164	0
	> 5 mm	0	0	165	0	*	0	665	0	0	6	0	25	0	0	0	0	151	0
	Total	0	0	165	0	*	0	665	0	0	6	0	25	0	0	0	0	315	0
	Dead snails	0	0	75	0	*	0	105	0	0	0	0	1	0	0	0	0	115	0
AUG	pH	7.6	7.3	7.4	7.9	7.4	6.2	6.7	6.9	6.3	7.7	8.6	7.7	7.9	8.1	7.8	7.7	5.8	7.1
	Temparature	32.1	30.0	29.6	31.1	30.8	36.0	36.7	36.1	33.6	37.7	37.9	33.3	32.3	39.1	29.6	27.3	27.9	29.9
(1991)	D. Oxygen	3.6	5.2	4.6	3.0	5.5	8.5	6.3	2.5	2.6	4.7	7.1	3.9	7.0	4.1	3.9	2.9	3.3	8.6
	EC	130.7	197.3	170.8	152.4	156.6	164.1	179.3	73.9	165.3	104.5	79.3	77.8	88.5	143.1	82.5	122.8	134.9	95.7
	Egg masses	++	-	+++	-	+++	-	+++	-	-	+	+	-	-	-	-	-	++	-
	< 5mm	3	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	45	0
	> 5 mm	32	0	225	0	114	0	985	0	0	9	13	0	5	0	0	0	35	0
	Total	35	0	225	0	125	0	985	0	0	9	13	0	5	0	0	0	80	0
	Dead snails	20	0	150	0	35	0	0	0	0	0	0	0	0	0	0	0	35	0
SEP	pH	7.4	7.5	7.4	7.4	7.4	7.5	4.1	7.5	7.3	7.2	7.2	7.3	7.9	7.4	7.7	7.6	7.9	7.2
	Temparature	34.4	32.7	31.6	35.4	30.4	29.9	30.4	32.2	28.8	35.1	32.2	30.2	29.0	33.2	27.2	28.3	30.3	33.3
(1991)	D. Oxygen	3.0	2.6	4.7	2.9	2.6	4.6	7.7	2.2	4.1	3.4	3.1	5.3	4.8	2.1	2.6	2.4	1.9	4.4
	EC	180.5	103.4	174.6	181.1	135.5	186.6	135.7	73.8	152.1	174.4	85.5	155.5	125.1	139.6	118.7	123.9	116.6	193.4
	Egg masses	-	-	-	+	+	-	+++	-	-	-	-	++	+++	-	-	-	+	-
	< 5mm	0	0	0	0	0	0	73	0	0	0	0	0	0	0	0	0	28	0
	> 5 mm	0	0	25	30	55	0	547	0	0	0	0	75	425	0	0	0	62	0
	Total	0	0	25	30	55	0	620	0	0	0	0	75	425	0	0	0	90	0
	Dead snails	0	0	0	15	15	0	20	0	0	0	0	15	35	0	0	0	30	0
OCT	pH	7.3	8.2	**	8.0	7.6	6.5	7.4	6.7	6.4	7.5	6.6	6.5	6.6	7.4	7.0	5.6	6.8	6.9
	Temparature	33.3	34.8	**	32.2	28.8	31.3	29.7	29.8	32.4	35.3	29.6	32.2	36.2	33.8	28.1	21.4	25.7	29.4
(1991)	D. Oxygen	3.5	4.2	**	3.5	3.5	4.1	4.6	4.1	4.3	4.2	2.6	4.5	4.1	2.2	4.4	4.1	4.5	4.2
	EC	128.5	178.3	**	163.8	164.4	135.6	132.4	85.3	162.5	152.3	75.5	126.5	115.5	163.8	155.1	139.2	106.1	149.9
	Egg masses	+	-	**	+	++	+	+	-	-	+	-	+	+-	-	-	-	++	-
	< 5mm	0	1	**	0	7	0	11	0	0	0	8	0	0	0	0	0	37	0
	> 5 mm	15	19	**	40	23	30	19	0	0	10	7	25	60	0	0	0	88	0
	Total	15	20	**	40	30	30	30	0	0	10	15	25	60	0	0	0	125	0
	Dead snails	20	1	**	1	40	5	10	0	0	5	2	11	45	0	0	0	45	0
NOV	pH	8.93	8.72	**	9.94	9.21	9.2	8.98	8.78	8.62	**	7.35	9.14	**	9.5	9.7	9.36	8.71	8.9
	Temparature	25.5	22	**	26.1	22.7	19.8	21.2	22.2	24.6	**	27.7	28.1	**	25.9	17.4	18.3	18.7	24.2
(1991)	D. Oxygen	4.3	2.6	**	4.1	4.4	5.1	3.6	3.7	2.1	**	2.7	1.1	**	3.6	2.4	2.8	4.5	3.9
	EC	166.4	166.9	**	120.4	136.2	122	177.5	100.6	159.7	**	79.9	161.5	**	179.2	198.5	156.1	144.7	171.7
	Egg masses	-	-	**	++	+	+	++	-	-	**	-	-	**	-	-	-	-	-
	< 5mm	0	3	**	0	5	0	0	0	0	**	0	0	**	0	0	0	0	0
	> 5 mm	110	3	**	175	55	150	250	0	0	**	8	0	**	0	0	0	0	0
	Total	110	3	**	175	60	150	250	0	0	**	8	0	**	0	0	0	0	0
	Dead snails	0	0	**	0	0	8	1	0	0	**	0	0	**	0	0	0	0	0
DEC	pH	9.13	8.82	**	9.31	8.58	9.52	9.96	9.61	9.44	**	9.82	9.1	8.85	8.89	9.46	9.9	8.6	9.43
	Temparature	17.9	23.8	**	23.9	18.6	18.4	16.8	21.2	22.7	**	27.9	25.2	27.7	26.6	14.9	15.6	21.7	23.3
(1991)	D. Oxygen	2.1	1.6	**	3.6	1.3	3.2	2.7	2.4	2.3	**	6.1	3.2	2.1	2.7	4.4	3.4	2.6	3.7
	EC	191.2	157.2	**	158.7	194.4	172.5	132.7	100.3	171.2	**	74.9	140.4	155.5	199.9	139.6	172.4	199.9	158.1
	Egg masses	++	-	**	+++	+++	+++	+++	-	-	**	-	-	-	-	-	-	-	-
	< 5mm	0	2	**	0	85	0	0	0	0	**	0	0	0	0	0	0	0	0
	> 5 mm	150	25	**	400	290	450	300	0	0	**	0	0	0	0	0	0	0	0
	Total	150	25	**	400	375	450	300	0	0	**	0	0	0	0	0	0	0	0
	Dead snails	0	2	**	0	0	3	2	0	0	**	0	0	0	0	0	0	0	0

Types of habitat: ICH = irrigation chanal; SC = seepage from canal; ICL = canal; DDW = ditches around drinking water place; RSP = road side pool; RVR = river.

Location: Tar = Tarahara; Bha = Bhantabari; Jhu = Jumka; Let = Letang.

Egg masses: +++ = abundant; ++ = moderate; + = scanty; - = absent.

* = habitats flooded; ** = habitats = dried up

Continued on next page

Appendix Table 4.2.4 (continued)

Month	Habitat No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	Habitat type	SC	DDW	RSP	ICH	RSP	RSP	RSP	RSP	RVR	RSP	RSP	DDW	ICL	RVR	DDW	ICH	RSP	RVR
	Location	Tar	Tar	Tar	Tar	Tar	Bha	Bha	Bha	Bha	Jhu	Jhu	Jhu	Jhu	Jhu	Let	Let	Let	Let
JAN	pH	8.95	9.15	**	9.82	9.3	9.88	10.73	10.28	9.45	**	9.4	9.66	9.38	9.53	6.5	9.73	9.11	9.82
	Temparature	23.2	26.3	**	22.3	23.5	22.5	21.9	21.8	22.5	**	28.2	22.5	21.1	23.3	15	16	17.9	28.6
(1992)	D. Oxygen	4	1.7	**	2.7	2.4	2.7	3.9	4.1	5.1	**	2.4	3.7	4.2	2.3	3.4	3.4	4.3	3
	EC	126.9	182.6	**	135.3	149.3	142.8	148.9	103.3	177.1	**	102.6	192.5	149.5	135.5	168.5	176.3	66.6	199.7
	Egg masses	+++	+++	**	+++	++	+++	++	-	-	**	-	-	-	-	+++	-	-	-
	< 5mm	15	11	**	28	12	15	28	0	0	**	0	0	0	0	54	0	0	0
	> 5 mm	555	189	**	852	150	235	220	0	0	**	0	0	0	0	150	0	0	0
	Total	570	200	**	880	162	250	248	0	0	**	0	0	0	0	204	0	0	0
	Dead snails	0	6	**	0	3	35	12	0	0	**	0	0	0	0	0	0	0	0
FEB	pH	9.58	9.3	**	9.3	9.78	9.54	9.34	9.83	9.47	**	9.26	9.43	9.39	9.53	9.97	9.81	9.38	8.9
	Temparature	17.9	17	**	26.3	16.9	19.5	22.7	23.9	23.6	**	27.1	24.4	26.6	27.2	15.1	17.1	20.2	22.5
(1992)	D. Oxygen	2	2.6	**	2.9	1.8	4.3	4	3.2	3.7	**	4.1	3.7	4.8	3.2	4.7	4	3.1	2.9
	EC	153.6	130.7	**	120.6	128.4	144.3	147.1	89.3	163.7	**	112.6	154.3	110.9	149.3	170.1	170.5	165.3	173.8
	Egg masses	++	++	**	++	-	++	+++	+	-	**	-	-	+	-	+++	-	+	-
	< 5mm	12	21	**	4	24	2	1	0	0	**	0	0	1	0	338	0	0	0
	> 5 mm	755	1015	**	117	11	40	140	35	0	**	0	0	19	0	488	0	4	0
	Total	767	1036	**	121	35	42	141	35	0	**	0	0	20	0	825	0	4	0
	Dead snails	12	0	**	40	5	125	60	4	0	**	0	0	0	0	0	0	0	0
MAR	pH	9.25	9.29	**	9.15	8.88	9.73	9.54	9.43	9.53	**	9.27	8.76	9.54	9.19	10.4	10.21	10.31	9.61
	Temparature	27.8	26.3	**	27.4	20.5	19.6	21.5	24.3	24.7	**	23.6	22.6	20.9	24.3	23.1	22.1	23.3	30.1
(1992)	D. Oxygen	1	2.1	**	1.7	2.1	4.3	5.7	4.2	4.1	**	3.9	5.2	4.1	3.1	5.5	6.1	4.8	3.8
	EC	164.5	142.2	**	139.9	121.3	154.7	185.5	96.4	103.2	**	120.5	186.9	139.9	149.9	140.8	187.5	162.4	130.1
	Egg masses	+++	+++	**	++	+	+	++	+	-	**	-	-	+	-	+++	-	+	-
	< 5mm	32	71	**	12	1	8	6	2	0	**	0	0	3	0	57	0	1	0
	> 5 mm	305	564	**	150	45	35	155	60	0	**	0	0	35	0	688	0	14	0
	Total	337	635	**	162	46	43	161	62	0	**	0	0	38	0	745	0	15	0
	Dead snails	15	75	**	60	30	20	45	7	0	**	0	0	2	0	12	0	0	0
APR	pH	9.12	9.6	**	8.93	9.47	10.7	10.9	9.81	10.22	**	9.4	9.43	9.13	9.63	10.48	10.29	9.91	9.38
	Temparature	27	25.9	**	25.9	26.7	23.5	22.2	29.4	22.6	**	32.4	35.5	34.1	31.3	22.9	24.1	25.3	26.3
(1992)	D. Oxygen	3.1	2.7	**	1.6	3	3.6	3.6	4.1	3.2	**	3.2	3.6	3.2	2.1	2	6	4.1	2.8
	EC	122.5	191.2	**	133.5	150.8	140.5	143.3	109.9	162.7	**	199.7	133.1	158.5	141.3	168.8	191.4	182.7	199.9
	Egg masses	+++	+++	**	+++	+++	-	++	+	-	**	+	-	+	-	-	-	++	-
	< 5mm	103	20	**	58	6	0	0	0	0	**	0	0	12	0	2	0	4	0
	> 5 mm	747	325	**	267	300	6	40	35	0	**	25	0	25	0	25	0	25	0
	Total	850	345	**	325	306	6	40	35	0	**	25	0	37	0	27	0	29	0
	Dead snails	25	17	**	49	7	8	65	14	0	**	7	0	0	0	43	0	0	0

Types of habitat: ICH = irrigation chanal; SC = seepage from canal; ICL = canal; DDW = ditches around drinking water place; RSP = road side pool; RVR = river.

Location: Tar = Tarahara; Bha = Bhantabari; Jhu = Jumka; Let = Letang.

Egg masses: +++ = abundant; ++ =moderate; + = scanty; - = absent.

** = habitats dried up.

Appendix Table 4.2.5

Seasonal changes in physical and chemical properties of water in *Lymnaea* habitats in the hills.

Month	Temperature °C				pH				Electro-conductivity (µs)				Dissolved oxygen (mg/l)			
	+ve for snails		-ve for snails		+ve for snails		-ve for snails		+ve for snails		-ve for snails		+ve for snails		-ve for snails	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Apr	20.1	1.0	18.1	6.4	6.9	0.2	6.8	2.4	50.9	6.0	35.9	12.7	2.6	0.4	0.7	0.2
May	22.6	1.1	17.9	6.3	7.0	0.2	6.6	2.3	56.4	4.2	36.7	13.0	1.8	0.3	-0.2	-0.1
Jun	23.1	0.6	19.5	6.2	6.9	0.1	6.7	2.1	51.0	3.7	51.3	16.2	1.6	0.3	-0.4	-0.1
Jul	25.2	1.1	18.9	0.4	7.0	0.1	7.1	0.1	47.7	5.3	32.6	2.5	1.9	0.4	0.3	0.6
Aug	24.7	0.9	20.8	1.4	7.0	0.0	7.1	0.0	63.8	5.8	51.1	4.0	2.5	0.3	1.6	0.3
Sep	21.7	0.7	21.5	1.4	6.9	0.0	7.0	0.0	72.5	5.2	66.2	5.6	1.9	0.2	1.4	0.3
Oct	24.0	1.5	20.9	1.1	7.3	0.2	7.3	0.2	44.1	3.9	32.7	4.3	2.8	0.4	3.1	0.6
Nov	17.1	1.1	17.5	0.9	7.1	0.1	7.1	0.1	53.0	5.4	55.7	8.2	2.1	0.2	2.3	0.3
Dec	17.5	1.0	15.8	0.8	7.2	0.0	7.1	0.0	57.7	4.2	46.5	4.9	2.0	0.2	5.3	0.3
Jan	17.6	1.1	14.5	0.7	7.0	0.1	6.8	0.1	63.8	7.5	47.6	4.1	1.8	0.3	1.9	0.3
Feb	14.7	0.7	14.9	0.8	6.9	0.1	6.8	0.1	64.3	4.9	46.5	2.8	1.7	0.2	1.4	0.2
Mar	18.5	1.3	16.5	0.8	7.3	0.0	7.2	0.0	64.1	4.9	44.9	4.1	1.9	0.2	1.5	0.3
Apr	20.2	1.2	18.0	1.5	6.7	0.1	7.0	0.2	73.4	5.9	51.1	3.8	1.9	0.2	1.2	0.3

Appendix Table 4.2.6

Seasonal changes in physical and chemical properties of water in *Lymnaea* habitats in the Terai.

Month	Temperature °C				pH				Electro-conductivity (µs)				Dissolved oxygen (mg/l)			
	+ve for snails		-ve for snails		+ve for snails		-ve for snails		+ve for snails		-ve for snails		+ve for snails		-ve for snails	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Apr	31.7	0.9	29.6	1.5	8.4	0.3	7.9	0.2	151.4	20.8	158.8	17.2	7.9	1.8	9.1	2.0
May	30.0	1.3	28.9	0.8	8.1	0.1	7.7	0.2	149.6	9.2	125.7	18.0	6.9	1.3	8.5	4.5
Jun	33.0	0.6	31.9	0.6	7.5	0.1	7.7	0.1	117.5	8.9	126.1	4.1	7.2	1.2	8.4	1.7
Jul	34.2	1.1	33.6	1.4	8.4	0.6	7.8	0.1	133.7	16.4	109.1	11.1	6.8	0.7	6.4	0.6
Aug	33.1	1.3	32.6	1.1	7.4	0.3	7.3	0.2	130.6	12.4	127.5	13.0	8.3	0.5	7.5	0.7
Sep	31.0	0.7	31.6	0.8	7.0	0.5	7.4	0.1	146.3	8.6	139.3	12.0	7.3	0.7	6.1	0.3
Oct	31.7	0.9	29.2	1.6	7.2	0.2	6.7	0.2	134.4	8.5	142.6	11.0	6.9	0.2	6.9	0.3
Nov	23.8	1.2	22.4	1.2	8.9	0.3	9.0	0.1	133.7	13.1	159.9	8.5	7.0	0.3	6.0	0.3
Dec	19.9	1.2	22.7	1.4	9.2	0.2	9.3	0.1	167.8	8.7	151.2	12.0	5.4	0.3	6.3	0.4
Jan	22.1	1.2	22.4	1.3	9.2	0.5	9.6	0.1	150.6	6.7	144.8	14.5	6.0	0.3	6.6	0.3
Feb	20.6	1.2	23.7	1.4	9.5	0.1	9.4	0.1	136.0	7.5	154.0	8.3	6.3	0.3	6.6	0.2
Mar	23.5	0.9	24.6	1.1	9.6	0.2	9.4	0.2	144.8	7.4	146.4	13.1	6.6	0.5	7.4	0.4
Apr	26.8	1.1	28.0	2.1	9.8	0.2	9.8	0.2	154.7	8.2	165.7	11.8	6.1	0.2	6.5	0.6

Appendix Table 4.2.7

Seasonal changes in population density of *Lymnaea* snails in the hills

Month	Habitats			Live snail population (per 5 man min.)						Egg masses		Empty shells	
	Size	Examined	+ ve for	Total		< 5 mm long		5 or > 5 mm long		(0-3 score)		(per 5 man min)	
	(sq m)	(No)	snails (No)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Apr	580.2	26	18	283.9	78.9	50.3	30.3	233.6	68.9	1.5	0.2	4.9	2.4
May	711.7	26	18	152.6	34.5	0.8	0.5	151.7	34.6	1.1	0.2	18.6	10.7
Jun	1153.1	26	16	140.8	31.6	15.4	8.6	125.4	28.5	0.5	0.2	2.6	0.8
Jul	1319.2	26	18	57.8	19.6	3.6	2.3	54.1	20.0	0.7	0.2	7.5	5.9
Aug	1297.3	26	14	25.4	7.5	0.7	0.3	26.7	7.4	0.7	0.2	1.8	1.0
Sep	1124.2	26	13	26.5	4.3	0.7	0.4	15.7	4.4	0.9	0.2	1.2	0.3
Oct	962.9	26	14	46.3	10.4	1.5	0.7	44.8	10.1	0.6	0.2	1.0	0.6
Nov	742.5	26	11	27.3	7.5	0.6	0.5	26.7	7.6	0.6	0.2	2.8	1.3
Dec	616.3	26	13	34.8	6.5	13.4	6.0	31.4	6.0	1.1	0.3	2.1	0.8
Jan	495.6	24	14	108.6	38.1	40.5	25.9	68.1	15.5	1.6	0.2	10.5	6.4
Feb	474.0	25	15	96.0	23.0	10.8	5.9	85.2	21.0	1.2	0.2	8.1	2.0
Mar	468.3	25	15	185.7	50.2	51.9	37.8	133.8	37.8	1.3	0.3	3.5	2.4
Apr	554.8	25	16	260.9	78.7	32.5	17.4	228.4	72.3	1.6	0.2	3.6	1.1

Appendix Table 4.2.8

Seasonal changes in population density of *Lymnaea* snails in the Terai

Month	Habitats			Live snail population (per 5 man min.)						Egg masses		Empty shells	
	Size	Examined	+ ve for	Total		< 5 mm long		5 or > 5 mm long		(0-3 score)		(per 5 man min)	
	(sq m)	(No)	snails (No)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Apr	NE	14	10	435.1	92.1	105.6	43.3	329.5	70.9	2.8	0.1	9.9	4.2
May	NE	16	8	319.9	53.0	35.3	15.9	284.6	57.8	2.6	0.1	26.9	7.9
Jun	NE	16	8	57.6	28.9	21.0	15.6	36.6	16.4	1.4	0.3	18.4	12.3
Jul	NE	17	5	235.2	108.2	32.9	29.4	202.3	107.4	1.8	0.5	59.2	22.2
Aug	NE	18	8	184.6	117.4	7.4	5.6	177.2	118.4	1.9	0.4	30.0	18.0
Sep	NE	18	7	188.6	82.6	14.4	9.7	174.2	75.8	1.6	0.4	18.6	4.0
Oct	NE	17	11	36.4	9.3	5.8	3.2	30.6	7.0	1.1	0.2	16.8	5.2
Nov	NE	15	6	125.5	32.0	0.8	0.8	124.7	32.3	1.2	0.3	1.5	1.2
Dec	NE	16	6	283.3	61.1	14.2	12.9	269.2	58.9	2.3	0.5	0.8	0.5
Jan	NE	16	7	359.1	93.5	23.4	5.4	335.8	93.5	2.7	0.2	8.0	4.4
Feb	NE	16	10	302.6	121.1	40.4	31.4	262.3	108.9	1.7	0.3	24.6	12.2
Mar	NE	16	10	204.0	78.4	17.6	7.5	186.4	71.2	1.9	0.3	24.2	7.7
Apr	NE	14	11	299.1	74.7	18.7	9.4	280.4	66.1	2.2	0.3	21.4	6.3

NE = not estimated

Appendix Table
Prevalence of *F. gigantica* and other infections in *Lymnaea* snails in the hills (1991-1992)

Month	Number of snails examined	*F. gigantica* infected snails: +ve for redia		+ve for redia & cercaria		Overall		Xiphidocercariae infected snails		*Chaetogaster* spp. infected snails	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Apr	441	0	0.00	0	0.00	0	0.00	86	19.50	15	3.40
May	461	1	0.22	1	0.22	2	0.43	104	22.56	18	3.90
Jun	336	1	0.30	1	0.30	2	0.60	97	28.87	28	8.33
Jul	236	1	0.42	3	1.27	4	1.69	29	12.29	12	5.08
Aug	210	1	0.48	2	0.95	3	1.43	43	20.48	17	8.10
Sep	214	0	0.00	3	1.40	3	1.40	32	14.95	15	7.01
Oct	330	1	0.30	9	2.73	10	3.03	38	11.52	25	7.58
Nov	269	1	0.37	5	1.86	6	2.23	80	29.74	32	11.90
Dec	208	1	0.48	6	2.88	7	3.37	29	13.94	28	13.46
Jan	298	1	0.34	5	1.68	6	2.01	99	33.22	19	6.38
Feb	354	0	0.00	2	0.56	2	0.56	37	10.45	24	6.78
Mar	323	0	0.00	0	0.00	0	0.00	12	3.72	26	8.05
Apr	597	0	0.00	0	0.00	0	0.00	96	16.08	38	6.37

Appendix Table
Prevalence of *F. gigantica* and other infections in *Lymnaea* snails in the Terai (1991-1992)

Month	Number of snails examined	*F. gigantica* infected snails: +ve for redia		+ve for redia & cercaria		Overall		Xiphidocercariae infected snails		*Chaetogaster* spp. infected snails	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Apr	437	1	0.23	1	0.23	2	0.46	16	3.66	2	0.46
May	387	1	0.26	3	0.78	4	1.03	4	1.03	4	1.03
Jun	297	2	0.67	3	1.01	5	1.68	8	2.69	6	2.02
Jul	209	1	0.48	3	1.44	4	1.91	3	1.44	1	0.48
Aug	294	0	0.00	2	0.68	2	0.68	7	2.38	0	0.00
Sep	331	0	0.00	6	1.81	6	1.81	8	2.42	3	0.91
Oct	471	0	0.00	6	1.27	6	1.27	12	2.55	4	0.85
Nov	259	0	0.00	2	0.77	2	0.77	9	3.47	3	1.16
Dec	251	1	0.40	2	0.80	3	1.20	6	2.39	4	1.59
Jan	314	0	0.00	1	0.32	1	0.32	8	2.55	4	1.27
Feb	318	0	0.00	1	0.31	1	0.31	9	2.83	5	1.57
Mar	417	1	0.24	2	0.48	3	0.72	6	1.44	3	0.72
Apr	378	1	0.26	2	0.53	3	0.79	8	2.12	3	0.79

Appendix Table 4.4.1
Cross-sectional Survey of Farm/Flock (January-February 1991)
Cattle in the hills

No	Age	Sex	Alti	Sys	EPG
1	1.0	M	1500	S	12
2	0.4	F	1475	S	0
3	11.0	M	1475	G	6
4	8.0	M	1475	G	6
5	5.0	F	1475	G	10
6	1.5	M	1475	G	10
7	2.5	M	1475	G	41
8	4.0	M	1475	G	4
9	5.0	F	1400	G	0
10	6.0	F	1400	G	0
11	9.0	M	1400	G	16
12	9.0	M	1400	G	11
13	5.0	F	1400	G	0
14	10.0	F	1400	S	0
15	5.0	M	1450	S	0
16	1.0	F	1450	S	0
17	3.0	F	1450	S	0
18	8.0	F	1450	S	0
19	1.5	M	1450	S	48
20	1.0	F	1450	S	3
21	11.0	F	1450	G	35
22	2.0	M	1450	G	0
23	2.0	F	1450	G	1
24	8.0	M	1450	G	3
25	10.0	F	1450	G	0
26	6.0	M	1450	G	33
27	6.0	M	1400	G	4
28	8.0	F	1400	G	26
29	9.0	M	1400	G	29
30	10.0	M	1400	G	0
31	4.0	F	1400	G	5
32	5.0	M	1425	G	0
33	3.0	F	1425	G	0
34	7.0	M	1425	G	0
35	1.0	F	1450	S	0
36	10.0	M	1450	G	0
37	6.0	M	1450	G	4
38	4.0	M	1400	G	48
39	4.0	M	1400	G	13
40	10.0	M	1400	G	0
41	3.0	F	1400	G	1
42	4.0	F	1400	G	0
43	6.0	F	1400	G	1
44	9.0	M	1400	G	0
45	10.0	M	1400	G	0
46	5.0	M	1400	G	0
47	1.0	M	1400	S	1
48	10.0	M	1500	G	5
49	7.0	M	1500	G	4
50	3.0	F	1500	G	1
51	9.0	M	1400	G	0
52	3.0	F	1400	G	1
53	1.5	M	1375	G	0
54	1.5	M	1375	G	8
55	3.0	F	1375	G	0
56	9.0	M	1375	G	0
57	4.0	M	1375	G	0
58	2.0	F	1375	G	0
59	5.0	F	1375	G	1
60	7.0	F	1375	G	27
61	5.0	M	1375	G	10
62	10.0	M	1375	G	3
63	9.0	F	1350	G	91
64	7.0	F	1350	G	0
65	8.0	F	1350	G	1
66	8.0	F	1350	G	112
67	2.0	M	1350	G	21
68	3.0	M	1350	G	7
69	10.0	F	1350	G	3
70	1.5	M	1350	G	20
71	3.0	M	1350	G	7
72	8.0	M	1350	G	0
73	1.0	M	1350	S	3
74	1.0	F	1350	S	0
75	12.0	M	1350	G	4
76	8.0	M	1350	G	4
77	1.5	F	1350	S	0
78	9.0	M	1350	G	0
79	6.0	F	1350	G	33
80	2.0	F	1350	G	51
81	2.0	F	1350	G	0
82	1.5	M	1350	S	7
83	1.0	F	1350	S	0
84	10.0	M	1350	G	8
85	5.0	M	1350	G	7
86	0.5	F	1350	S	0
87	9.0	F	1375	G	1
88	3.0	F	1375	G	38
89	0.6	M	1375	S	7
90	1.0	M	1375	S	35
91	9.0	F	1375	G	0
92	9.0	M	1375	G	18
93	8.0	F	1375	G	9
94	9.0	M	1375	G	0
95	5.0	M	1375	G	8
96	2.0	F	1375	G	10
97	4.0	F	1375	G	21
98	11.0	F	1375	G	0
99	1.5	M	1375	G	1
100	7.0	M	1350	G	0
101	7.0	F	1350	G	4
102	7.0	F	1350	G	4
103	3.0	M	1350	G	4
104	2.0	F	1350	G	1
105	10.0	M	1350	G	0
106	8.0	F	1350	G	0
107	1.5	F	1350	S	36
108	6.0	F	1350	G	66
109	8.0	M	1350	G	64
110	2.0	M	1350	G	69
111	1.0	F	1375	S	27
112	3.0	F	1375	G	48
113	3.0	M	1375	G	5
114	4.0	M	1375	G	20
115	4.0	F	1400	G	121
116	9.0	M	1400	G	26
117	7.0	F	1400	S	0
118	6.0	M	1300	G	83
119	2.0	M	1300	G	4
120	9.0	F	1300	G	3
121	8.0	M	1300	G	7
122	1.5	M	1300	S	8
123	7.0	F	1300	G	1
124	9.0	M	1300	G	3
125	7.0	F	1300	G	27
126	1.5	M	1300	G	35
127	7.0	M	1300	G	0
128	1.5	F	1300	S	1
129	7.0	F	1300	G	12
130	4.0	M	1300	G	0
131	6.0	M	1325	G	13
132	1.5	F	1325	S	18
133	0.6	F	1325	G	0
134	2.0	F	1325	G	18
135	7.0	M	1325	G	66
136	3.0	F	1325	G	14
137	9.0	M	1325	G	1
138	1.5	F	1300	S	0
139	5.0	F	1300	G	0
140	9.0	F	1300	G	0
141	3.0	F	1300	G	0
142	2.0	F	1300	G	0
143	7.0	F	1300	G	10
144	6.0	M	1300	G	18
145	6.0	M	1300	G	0
146	6.0	F	1300	G	7
147	10.0	M	1300	S	1
148	5.0	F	1300	G	0
149	2.0	F	1300	G	0
150	7.0	M	1300	G	3
151	9.0	M	1300	G	0
152	8.0	F	1300	G	3
153	9.0	F	1300	G	13
154	1.5	F	1300	S	1
155	6.0	M	1300	G	4
156	6.0	M	1300	G	4
157	10.0	F	1300	G	0
158	3.0	F	1300	G	0
159	6.0	M	1300	G	0
160	1.5	M	1300	G	0
161	2.0	M	1325	G	0
162	10.0	F	1325	G	0
163	8.0	M	1325	G	3
164	5.0	F	1325	G	18
165	9.0	M	1325	G	0
166	3.0	F	1325	G	0
167	2.0	M	1325	G	0
168	7.0	F	1325	G	0
169	8.0	M	1325	G	4
170	0.5	M	1325	S	0
171	3.0	F	1325	G	0
172	4.0	F	1325	G	0
173	5.0	M	1325	G	0
174	6.0	F	1325	G	0
175	3.0	F	1300	G	0
176	7.0	M	1300	G	0
177	2.0	F	1300	G	0
178	4.0	F	1300	G	0
179	2.0	F	1300	G	1
180	3.0	F	1200	G	10
181	3.0	M	1200	G	0
182	9.0	F	1200	G	0
183	5.0	F	1200	G	0
184	2.0	F	1200	G	0
185	9.0	F	1200	G	1
186	8.0	M	1200	G	5
187	1.5	F	1225	G	0
188	10.0	F	1225	G	14
189	9.0	M	1225	G	1
190	3.0	F	1225	G	3
191	4.0	M	1225	G	1
192	6.0	M	1250	G	0
193	9.0	F	1250	G	0
194	6.0	M	1250	G	0
195	2.0	F	1250	G	0
196	1.5	F	1225	G	5
197	5.0	F	1225	G	3
198	4.0	M	1225	G	0
199	2.0	F	1225	G	5
200	5.0	M	1225	G	0
201	8.0	F	1225	G	4
202	8.0	M	1225	G	0
203	2.0	F	1225	G	0
204	4.0	F	1225	G	0
205	8.0	M	1225	G	0
206	2.0	M	1250	G	29
207	12.0	F	1250	G	0
208	12.0	F	1250	G	0
209	3.0	M	1250	G	0
210	2.0	M	1250	G	10
211	9.0	M	1250	G	3
212	4.0	F	1250	G	3
213	12.0	M	1250	G	0
214	3.0	F	1250	G	16
215	5.0	M	1250	G	4
216	2.0	F	1250	G	5
217	8.0	M	1225	G	5
218	7.0	F	1225	G	5
219	8.0	M	1225	G	4
220	2.5	M	1250	G	10
221	2.5	F	1250	G	0
222	1.5	M	1300	G	0
223	6.0	M	1400	G	0
224	8.0	F	1400	G	100
225	5.0	M	1400	G	0
226	8.0	F	1400	G	3
227	6.0	M	1400	G	26
228	2.0	F	1400	G	1
229	2.5	F	1400	G	13
230	7.0	M	1400	G	0
231	2.0	M	1400	G	7
232	8.0	F	1400	G	29
233	8.0	F	1400	G	5
234	8.0	M	1400	G	25
235	9.0	F	1400	G	3
236	8.0	M	1400	G	35
237	4.0	F	1400	G	4
238	3.0	F	1400	G	59
239	3.0	F	1400	G	63
240	8.0	M	1400	G	3
241	9.0	M	1400	G	66
242	5.0	M	1400	G	66
243	8.0	M	1400	G	0
244	4.0	M	1400	G	4
245	2.5	F	1400	G	0
246	0.5	M	1225	S	0
247	1.5	F	1225	S	0
248	8.0	M	1225	G	0
249	6.0	F	1225	S	0
250	10.0	F	1250	G	3
251	1.0	F	1250	G	0
252	4.0	F	1250	G	1
253	7.0	M	1250	G	18
254	6.0	F	1250	G	0
255	5.0	F	1100	G	0
256	3.0	F	1100	G	0
257	9.0	F	1100	G	3
258	9.0	M	1100	G	8
259	7.0	M	1100	G	0
260	3.0	F	1100	G	0
261	2.0	F	1100	G	1
262	8.0	M	1100	G	13
263	4.0	F	1000	G	0
264	8.0	M	1000	G	0
265	2.5	F	1000	G	5
266	4.0	M	1000	G	17
267	8.0	M	1000	G	22
268	3.0	M	1000	G	0
269	9.0	F	1050	G	5
270	2.0	M	1050	G	0
271	3.0	F	1050	G	1
272	2.0	F	1050	G	0
273	8.0	F	1050	G	0
274	5.0	M	1050	G	0
275	7.0	F	1100	G	0
276	3.0	F	1100	G	0
277	8.0	F	1100	G	0
278	2.0	F	1100	G	0
279	3.0	F	1100	G	0
280	3.0	F	1100	G	0
281	8.0	F	1100	G	0
282	9.0	M	1100	G	0
283	5.0	M	1250	G	18
284	7.0	M	1250	G	0
285	5.0	M	1250	G	25
286	5.0	M	1250	G	7
287	3.0	F	1250	G	0
288	9.0	M	1250	G	1
289	9.0	M	1200	G	5
290	8.0	F	1200	G	42
291	6.0	F	1200	G	1
292	2.0	M	1200	G	1
293	6.0	M	1250	G	3
294	3.0	F	1250	G	0
295	1.0	F	1250	S	4
296	4.0	F	1250	G	1
297	3.0	F	850	G	0
298	1.0	F	850	G	0
299	8.0	M	850	G	0
300	10.0	M	850	G	0
301	9.0	M	850	S	5
302	1.5	F	850	G	0
303	5.0	F	850	G	0
304	4.0	F	850	G	0
305	3.0	M	825	G	14
306	2.0	M	825	G	8
307	4.0	F	825	G	0
308	4.0	F	825	G	0
309	5.0	M	825	G	0
310	9.0	F	825	G	0
311	2.0	F	825	G	0
312	0.8	F	825	S	0
313	9.0	F	825	G	4
314	6.0	F	825	G	0
315	7.0	M	825	G	0
316	8.0	M	825	S	0
317	1.5	F	825	S	0
318	1.5	F	825	G	0
319	5.0	F	825	G	0
320	5.0	M	825	G	4
321	2.0	F	825	G	1
322	2.0	F	825	G	0
323	8.0	F	825	G	0
324	8.0	F	850	G	0
325	3.0	F	850	G	0
326	1.5	F	850	G	0
327	5.0	F	850	G	0
328	8.0	F	825	G	0
329	4.0	F	825	G	0
330	2.0	F	825	G	0
331	11.0	M	825	G	0
332	2.0	M	825	G	0
333	2.0	M	825	G	0
334	7.0	F	825	G	0
335	6.0	F	825	G	0
336	6.0	F	825	G	0
337	9.0	M	825	G	0
338	4.0	F	800	G	0
339	4.0	F	800	G	0
340	5.0	F	800	G	0
341	9.0	M	800	G	0
342	3.0	F	850	G	0
343	14.0	F	850	G	4
344	5.0	F	850	G	6
345	16.0	M	850	G	0
346	7.0	F	850	G	0
347	1.0	F	850	S	0
348	11.0	F	800	G	0
349	10.0	M	800	G	0
350	1.0	F	800	S	0
351	7.0	F	800	G	4
352	1.5	M	800	G	4
353	2.0	F	800	G	2
354	1.5	M	800	G	2
355	6.0	F	800	G	0
356	5.0	M	800	G	18
357	7.0	F	800	G	2
358	3.0	F	800	G	0
359	6.0	M	800	G	0
360	6.0	F	800	G	22
361	10.0	F	875	G	2
362	0.7	M	875	G	0
363	2.5	M	875	G	0
364	3.0	F	875	G	4
365	6.0	M	875	G	0
366	0.8	F	875	G	0
367	0.9	F	875	S	0
368	3.0	F	875	G	2
369	4.0	M	875	G	2
370	4.0	M	875	G	0
371	7.0	M	875	G	0
372	0.5	F	875	S	0
373	2.0	F	875	G	0
374	2.0	M	875	G	2
375	8.0	F	875	G	18
376	2.0	F	800	G	0
377	4.0	M	800	G	4
378	5.0	F	800	G	0
379	5.0	F	800	G	14
380	4.0	F	650	G	2
381	6.0	M	650	G	22
382	0.5	F	650	G	0
383	12.0	F	650	G	0
384	0.9	M	650	S	0
385	9.0	F	650	G	0
386	3.0	M	650	G	0
387	9.0	F	650	G	0
388	6.0	M	650	G	0
389	8.0	M	650	G	0
390	6.0	M	650	G	2
391	3.0	F	650	G	0
392	3.0	M	650	G	0
393	8.0	F	650	G	0
394	2.0	M	450	G	0
395	4.0	F	450	G	0
396	11.0	F	450	G	0
397	8.0	M	450	G	0
398	4.0	M	450	G	0
399	8.0	F	450	G	0
400	4.0	M	450	G	0
401	7.0	F	450	G	0
402	3.0	M	450	G	0
403	8.0	F	450	G	0
404	2.0	M	450	G	0
405	2.0	F	450	G	16
406	11.0	M	450	G	0
407	5.0	M	450	G	0
408	3.0	F	450	G	4
409	7.0	F	450	G	2
410	10.0	F	450	G	20
411	0.7	F	450	S	0
412	5.0	M	450	G	10
413	6.0	M	450	G	6
414	1.5	M	750	G	0
415	1.5	F	750	G	0
416	9.0	F	750	G	0
417	2.0	M	750	G	0
418	11.0	M	750	G	0
419	10.0	F	750	G	0
420	2.0	F	650	G	2
421	2.0	M	650	G	0
422	14.0	M	650	G	0
423	11.0	F	650	G	0
424	8.0	M	650	G	4
425	2.0	M	650	G	0
426	8.0	F	700	G	0
427	9.0	M	700	G	0
428	6.0	F	700	G	0
429	11.0	F	700	G	0
430	1.0	M	700	S	0
431	10.0	M	650	G	0
432	4.0	M	650	G	0
433	8.0	F	650	G	0
434	1.0	F	650	S	0
435	14.0	F	650	G	0
436	2.0	M	650	G	0
437	15.0	M	650	G	0
438	4.0	F	650	G	0
439	3.0	M	650	G	0
440	10.0	F	650	G	2
441	1.0	F	650	S	0
442	4.0	M	650	G	0
443	12.0	M	650	G	0
444	0.4	F	650	G	0
445	8.0	F	650	G	0
446	6.0	F	650	G	0
447	9.0	F	650	G	0
448	2.0	F	650	G	0
449	5.0	M	650	G	2
450	11.0	F	650	G	0
451	2.0	M	650	G	0
452	7.0	M	650	G	4
453	5.0	F	650	G	6
454	3.0	M	650	G	4
455	2.0	F	650	G	2
456	2.0	M	630	G	0
457	2.0	F	630	G	0
458	3.0	M	630	G	2
459	15.0	F	630	G	0
460	10.0	F	630	G	0
461	1.0	M	550	S	0
462	2.0	M	550	G	0
463	4.0	F	550	G	4
464	11.0	F	550	G	0
465	14.0	M	550	G	0
466	10.0	F	600	G	2
467	9.0	M	600	G	0
468	9.0	F	600	G	0

No = Animal number; Age = age of animal in years; Alti = elevation (in metre) of the farm; Sys = management system, S = stall fed, G = out grazed, EPG = eggs per gram of faeces, M = male, F = female.

Appendix Table 4.4.1 (continued)

No	Age	Sex	Alti	Sys	EPG	No	Age	Sex	Alti	Sys	EPG	No	Age	Sex	Alti	Sys	EPG	No	Age	Sex	Alti	Sys	EPG
469	2.0	F	600	G	4	549	4.0	M	1700	G	2	629	4.0	M	1750	G	0	709	12.0	F	1750	G	0
470	2.0	F	600	G	0	550	14.0	F	1750	G	4	630	5.0	M	1750	G	0	710	6.0	F	1750	G	0
471	3.0	M	600	G	0	551	8.0	M	1750	G	0	631	3.0	F	1750	G	0	711	7.0	F	1750	G	0
472	10.0	M	600	G	0	552	7.0	M	1750	G	0	632	4.0	M	1750	G	0	712	5.0	M	1750	G	2
473	8.0	F	600	G	0	553	0.6	F	1750	G	4	633	5.0	M	1750	G	0	713	5.0	F	1750	G	4
474	2.0	M	600	G	0	554	10.0	F	1750	G	0	634	5.0	M	1750	G	8	714	7.0	F	1750	G	2
475	10.0	M	500	G	10	555	7.0	F	1750	G	0	635	7.0	F	1750	G	16	715	5.0	M	1750	G	0
476	9.0	M	500	G	0	556	7.0	F	1750	G	0	636	5.0	F	1750	G	0	716	1.0	F	1750	G	0
477	8.0	M	500	G	0	557	10.0	F	1750	G	4	637	4.0	F	1750	G	0	717	2.0	F	1750	G	0
478	2.0	F	500	G	0	558	3.0	M	1750	G	0	638	3.0	M	1750	G	0	718	9.0	M	1750	G	0
479	0.7	F	500	S	0	559	0.5	M	1750	G	0	639	7.0	F	1750	G	0	719	9.0	M	1750	G	0
480	0.7	F	500	G	0	560	7.0	M	1700	G	0	640	3.0	F	1750	G	0	720	1.5	M	1750	G	6
481	0.5	F	500	S	0	561	3.0	M	1700	G	0	641	8.0	F	1775	G	0	721	0.6	M	1750	G	0
482	12.0	F	500	G	0	562	10.0	F	1700	G	0	642	6.0	F	1775	G	0	722	7.0	F	1750	G	0
483	6.0	M	500	G	31	563	8.0	M	1550	G	0	643	3.0	F	1800	G	0	723	3.0	F	1750	G	0
484	6.0	M	500	G	0	564	8.0	F	1550	G	0	644	2.5	F	1800	G	0	724	12.0	F	1750	G	0
485	11.0	F	550	G	0	565	1.0	F	1550	G	2	645	2.5	F	1750	G	0	725	10.0	F	1750	G	2
486	7.0	M	550	G	0	566	6.0	M	1550	G	4	646	8.0	F	1750	G	4	726	1.0	M	1750	G	0
487	10.0	F	550	G	0	567	6.0	M	1550	G	0	647	1.5	M	1750	G	0	727	12.0	F	1750	G	18
488	1.0	M	550	S	0	568	4.0	M	1550	G	0	648	1.0	M	1750	G	0	728	1.5	F	1750	G	0
489	4.0	F	550	G	12	569	3.0	M	1550	G	2	649	7.0	M	1750	G	4	729	8.0	M	1750	G	39
490	3.0	F	550	G	6	570	8.0	F	1550	G	0	650	7.0	M	1750	G	0	730	5.0	M	1750	G	0
491	3.0	F	575	G	0	571	8.0	F	1600	G	0	651	2.0	F	1750	G	0	731	7.0	F	1800	G	0
492	7.0	F	575	G	0	572	4.0	F	1600	G	0	652	10.0	M	1750	G	12	732	8.0	F	1800	G	0
493	11.0	M	575	G	0	573	8.0	M	1700	G	2	653	10.0	M	1750	G	0	733	8.0	F	1800	G	0
494	10.0	F	525	G	2	574	10.0	M	1700	G	0	654	10.0	F	1750	G	4	734	8.0	F	1800	G	0
495	1.0	F	525	S	0	575	5.0	F	1700	G	2	655	1.5	M	1750	G	0	735	6.0	F	1800	G	0
496	1.5	F	525	G	0	576	10.0	F	1700	G	2	656	4.0	F	1750	G	0	736	5.0	F	1800	G	0
497	8.0	F	525	G	61	577	5.0	M	1700	G	2	657	5.0	M	1750	G	4	737	8.0	F	1800	G	0
498	9.0	M	525	G	2	578	7.0	M	1750	G	0	658	5.0	M	1750	G	0	738	5.0	F	1800	G	2
499	7.0	M	525	G	18	579	3.0	F	1750	G	0	659	5.0	F	1750	G	0	739	6.0	F	1800	G	0
500	9.0	F	550	G	4	580	12.0	F	1750	G	0	660	0.7	M	1750	G	0	740	9.0	F	1800	G	0
501	2.0	F	550	G	2	581	5.0	M	1750	G	0	661	8.0	F	1850	G	0	741	5.0	F	1800	G	0
502	10.0	M	550	G	2	582	5.0	M	1750	G	0	662	4.0	M	1850	G	0	742	4.0	F	1800	G	0
503	8.0	F	450	G	0	583	12.0	F	1750	G	0	663	4.0	M	1850	G	0	743	4.0	F	1900	G	0
504	0.8	M	450	G	6	584	6.0	M	1750	G	0	664	6.0	M	1850	G	0	744	2.0	M	1900	G	0
505	13.0	F	400	G	0	585	5.0	M	1750	G	0	665	4.0	M	1850	G	0	745	6.0	F	1900	G	0
506	3.0	F	400	G	2	586	7.0	F	1750	G	0	666	5.0	M	1850	G	0	746	12.0	F	1900	G	0
507	3.0	F	400	G	0	587	4.0	M	1750	G	2	667	5.0	M	1850	G	0	747	8.0	F	1900	G	0
508	2.0	M	400	G	0	588	4.0	M	1750	G	6	668	8.0	M	1850	G	10	748	13.0	M	1900	G	0
509	2.0	M	400	G	25	589	5.0	M	1550	G	8	669	8.0	M	1850	G	16	749	4.0	M	1900	G	0
510	9.0	F	400	G	2	590	5.0	M	1550	G	0	670	8.0	M	1800	G	0	750	6.0	F	1900	G	0
511	10.0	F	400	G	0	591	1.6	M	1550	G	0	671	9.0	M	1800	G	0	751	3.0	F	1900	G	0
512	15.0	M	400	G	0	592	13.0	M	1550	G	0	672	2.5	M	1800	G	0	752	10.0	F	1900	G	0
513	1.0	F	400	S	0	593	15.0	M	1550	G	0	673	10.0	F	1800	G	45	753	3.0	F	1900	G	0
514	7.0	M	400	G	0	594	14.0	F	1550	G	0	674	2.5	M	1800	G	8	754	2.0	M	1900	G	0
515	9.0	F	400	G	0	595	2.0	M	1550	G	0	675	10.0	F	1800	G	10	755	4.0	F	1900	G	0
516	5.0	M	350	G	0	596	5.0	M	1550	G	0	676	9.0	F	1750	G	0	756	7.0	F	1900	G	0
517	3.0	F	350	G	0	597	8.0	F	1600	G	2	677	4.0	F	1750	G	0	757	12.0	F	1900	G	0
518	11.0	M	400	G	2	598	2.0	F	1600	G	0	678	9.0	F	1750	G	20	758	5.0	F	1900	G	0
519	0.9	M	400	S	0	599	4.0	M	1600	G	0	679	16.0	M	1750	G	0	759	4.0	F	1900	G	0
520	4.0	M	400	G	4	600	5.0	M	1600	G	2	680	10.0	M	1750	G	2	760	2.0	M	1900	G	0
521	9.0	M	400	G	0	601	5.0	F	1700	G	0	681	7.0	M	1750	G	35	761	3.0	M	1900	G	0
522	10.0	M	650	G	2	602	8.0	M	1700	G	2	682	4.0	M	1750	G	0	762	6.0	F	1900	G	0
523	8.0	M	650	G	8	603	8.0	M	1700	G	4	683	6.0	F	1750	G	0	763	3.0	M	1900	G	0
524	9.0	F	650	G	4	604	10.0	F	1700	G	0	684	6.0	F	1750	G	2	764	8.0	F	1900	G	0
525	1.5	F	650	G	0	605	9.0	F	1700	G	0	685	2.0	M	1750	G	0	765	6.0	M	1900	G	0
526	1.5	M	650	G	4	606	5.0	F	1700	G	0	686	1.0	F	1750	G	0	766	10.0	M	1900	G	0
527	2.0	M	650	G	0	607	7.0	F	1700	G	2	687	5.0	F	1750	G	0	767	5.0	F	2000	G	0
528	4.0	F	650	G	0	608	6.0	F	1750	G	0	688	4.0	F	1750	G	0	768	7.0	F	2000	G	2
529	8.0	F	650	G	0	609	5.0	M	1750	G	6	689	2.0	F	1750	G	4	769	8.0	F	2400	G	0
530	10.0	F	650	G	2	610	6.0	M	1750	G	6	690	7.0	M	1750	G	0	770	7.0	F	2400	G	0
531	12.0	M	650	G	0	611	3.0	M	1750	G	8	691	7.0	M	1750	G	0	771	10.0	F	2400	G	0
532	12.0	F	600	G	0	612	12.0	F	1750	G	10	692	9.0	F	1750	G	6	772	12.0	F	2400	G	0
533	3.0	F	600	G	0	613	3.0	F	1750	G	0	693	12.0	F	1750	G	4	773	2.0	F	2400	G	0
534	3.0	M	600	G	0	614	2.0	F	1750	G	8	694	4.0	M	1750	G	0	774	3.0	F	2400	G	0
535	3.0	M	600	G	0	615	11.0	F	1750	G	0	695	5.0	M	1750	G	0	775	6.0	F	2400	G	12
536	13.0	F	600	G	0	616	6.0	M	1750	G	0	696	2.0	M	1750	G	6	776	6.0	F	2400	G	0
537	7.0	M	1600	G	4	617	6.0	M	1750	G	0	697	6.0	M	1750	G	0	777	8.0	F	2300	G	0
538	4.0	M	1600	G	0	618	3.0	M	1750	G	0	698	6.0	M	1750	G	16	778	9.0	F	2300	G	0
539	3.0	F	1600	G	0	619	7.0	F	1750	G	4	699	5.0	F	1750	G	0	779	1.0	M	2300	G	0
540	7.0	F	1700	G	14	620	2.0	M	1750	G	2	700	2.0	M	1750	G	0	780	5.0	F	2300	G	0
541	2.0	F	1700	G	0	621	9.0	F	1750	G	2	701	5.0	M	1750	G	2	781	7.0	F	2300	G	0
542	11.0	F	1700	G	0	622	7.0	F	1750	G	4	702	4.0	M	1750	G	12	782	2.0	M	2150	G	0
543	4.0	M	1700	G	0	623	6.0	M	1750	G	6	703	5.0	F	1750	G	14	783	10.0	F	2150	G	0
544	5.0	F	1650	G	0	624	7.0	M	1750	G	0	704	5.0	F	1750	G	0	784	6.0	F	2900	G	0
545	5.0	F	1650	G	0	625	10.0	M	1750	G	0	705	15.0	F	1750	G	8	785	4.0	F	2900	G	0
546	4.0	F	1650	G	8	626	6.0	M	1750	G	0	706	4.0	M	1750	G	0	786	8.0	F	2900	G	4
547	9.0	F	1650	G	2	627	3.0	F	1750	G	6	707	6.0	M	1750	G	2						
548	5.0	M	1700	G	0	628	7.0	F	1750	G	0	708	6.0	M	1750	G	2						

No =Animal number; Age = age of animal in years; Alti =elevation (in metre) of the farm; Sys =management system, S = stall fed, G =out grazed; EPG =eggs per gram of faeces; M =male, F = female.

Appendix Table 4.4.2
Farm/Flock's Cross-sectional Survey (March 1991)
Callte in the Terai

No	Age	Sex	Area	EPG
1	3.0	F	Tara	1
2	3.0	M	Tara	0
3	6.0	F	Tara	3
4	5.0	M	Tara	0
5	6.0	M	Tara	0
6	2.0	M	Tara	0
7	8.0	F	Tara	4
8	5.0	M	Tara	0
9	6.0	M	Tara	0
10	1.0	M	Tara	0
11	2.0	M	Tara	0
12	2.0	M	Tara	0
13	7.0	F	Tara	0
14	8.0	M	Tara	1
15	7.0	M	Tara	0
16	9.0	F	Tara	0
17	3.0	F	Tara	4
18	2.0	F	Tara	0
19	3.0	M	Tara	14
20	3.0	M	Tara	8
21	12.0	F	Tara	10
22	9.0	M	Tara	5
23	9.0	M	Tara	3
24	6.0	F	Tara	4
25	1.0	M	Tara	8
26	8.0	F	Tara	1
27	0.5	M	Tara	0
28	0.5	F	Tara	0
29	3.0	F	Tara	0
30	3.0	F	Tara	0
31	3.0	F	Tara	0
32	3.0	F	Tara	0
33	4.0	M	Tara	0
34	14.0	F	Tara	0
35	3.0	M	Tara	1
36	3.0	M	Tara	0
37	4.0	F	Tara	0
38	5.0	F	Tara	0
39	1.0	M	Tara	0
40	10.0	F	Tara	4
41	1.5	M	Tara	1
42	2.0	F	Tara	8
43	3.0	F	Tara	4
44	10.0	F	Tara	0
45	4.0	F	Tara	1
46	6.0	M	Tara	0
47	1.5	M	Tara	0
48	2.0	M	Tara	0
49	8.0	F	Tara	0
50	1.5	F	Tara	0
51	6.0	F	Tara	0
52	12.0	F	Tara	0
53	3.0	F	Tara	0
54	3.0	F	Tara	0
55	0.6	M	Tara	0
56	7.0	F	Tara	0
57	12.0	F	Tara	0
58	2.0	M	Tara	0
59	1.5	F	Tara	0
60	4.0	F	Tara	0
61	4.0	F	Tara	0
62	15.0	F	Tara	0
63	5.0	F	Tara	0
64	3.0	F	Tara	0
65	9.0	F	Tara	1
66	10.0	M	Tara	3
67	8.0	M	Tara	1
68	3.0	M	Tara	0
69	2.0	F	Tara	0
70	8.0	M	Tara	3
71	10.0	M	Tara	0
72	1.0	M	Tara	0
73	8.0	F	Tara	5
74	3.0	F	Tara	0
75	8.0	M	Tara	1
76	7.0	M	Tara	3
77	3.0	F	Tara	0
78	2.0	F	Tara	1
79	12.0	F	Tara	5
80	3.0	F	Tara	0
81	1.5	M	Tara	0
82	1.5	M	Tara	0
83	4.0	F	Tara	0
84	10.0	F	Tara	0
85	1.5	M	Tara	0
86	1.0	M	Tara	0
87	4.0	F	Tara	0
88	3.0	M	Tara	0
89	7.0	F	Tara	0
90	5.0	F	Tara	0
91	3.0	M	Tara	0
92	3.0	M	Tara	0
93	2.0	M	Tara	0
94	5.0	F	Tara	0
95	15.0	M	Tara	1
96	[illegible]	M	Tara	0
97	[illegible]	M	Tara	0
98	8.0	F	Tara	0
99	3.0	F	Tara	1
100	[illegible]	M	Tara	1
101	9.0	M	Tara	0
102	12.0	F	Tara	0
103	5.0	M	Tara	0
104	4.0	M	Tara	1
105	4.0	F	Tara	0
106	[illegible]	F	Tara	0
107	7.0	F	Tara	0
108	1.0	M	Tara	4
109	8.0	F	Tara	0
110	0.5	M	Tara	0
111	3.0	F	Tara	0
112	0.5	M	Tara	13
113	7.0	F	Tara	5
114	4.0	M	Tara	0
115	10.0	F	Tara	5
116	3.0	M	Tara	0
117	1.5	M	Tara	0
118	20.0	F	Tara	0
119	12.0	F	Tara	0
120	10.0	M	Tara	0
121	9.0	F	Tara	0
122	6.0	M	Tara	0
123	2.0	M	Tara	0
124	10.0	M	Tara	1
125	8.0	M	Tara	0
126	10.0	M	Tara	1
127	1.0	F	Tara	0
128	2.0	F	Tara	0
129	2.0	F	Tara	1
130	0.5	M	Tara	5
131	7.0	F	Tara	0
132	7.0	M	Tara	0
133	7.0	M	Tara	0
134	9.0	M	Tara	3
135	8.0	M	Tara	3
136	12.0	M	Tara	4
137	10.0	M	Tara	5
138	10.0	M	Tara	0
139	1.0	F	Tara	0
140	14.0	M	Tara	0
141	1.0	F	Tara	0
142	10.0	F	Tara	1
143	12.0	F	Tara	3
144	8.0	F	Tara	1
145	8.0	M	Bhan	14
146	12.0	F	Bhan	3
147	5.0	F	Bhan	1
148	5.0	M	Bhan	0
149	7.0	F	Bhan	0
150	2.5	F	Bhan	30
151	14.0	M	Bhan	0
152	8.0	F	Bhan	3
153	7.0	F	Bhan	9
154	6.0	F	Bhan	4
155	4.0	F	Bhan	0
156	1.0	M	Bhan	0
157	8.0	M	Bhan	0
158	7.0	M	Bhan	0
159	6.0	F	Bhan	4
160	12.0	M	Bhan	0
161	7.0	F	Bhan	0
162	6.0	F	Bhan	0
163	6.0	F	Bhan	0
164	9.0	M	Bhan	0
165	10.0	F	Bhan	0
166	9.0	M	Bhan	0
167	3.0	F	Bhan	0
168	1.0	M	Bhan	0
169	10.0	F	Bhan	0
170	5.0	F	Bhan	0
171	0.5	F	Bhan	0
172	15.0	M	Bhan	4
173	10.0	F	Bhan	13
174	5.0	F	Bhan	3
175	15.0	M	Bhan	1
176	15.0	M	Bhan	5
177	15.0	M	Bhan	3
178	13.0	F	Bhan	0
179	2.5	F	Bhan	20
180	15.0	M	Bhan	7
181	1.0	M	Bhan	9
182	10.0	F	Bhan	16
183	12.0	M	Bhan	7
184	2.5	M	Bhan	9
185	3.0	F	Bhan	5
186	6.0	M	Bhan	4
187	5.0	M	Bhan	8
188	1.0	M	Bhan	5
189	5.0	F	Bhan	5
190	5.0	F	Bhan	13
191	3.0	F	Bhan	0
192	1.5	M	Bhan	3
193	4.0	F	Bhan	0
194	6.0	F	Bhan	0
195	5.0	F	Bhan	4
196	4.0	F	Bhan	5
197	3.0	M	Bhan	0
198	5.0	F	Bhan	0
199	5.0	F	Bhan	0
200	15.0	M	Bhan	7
201	15.0	M	Bhan	0
202	8.0	F	Bhan	0
203	12.0	M	Bhan	14
204	11.0	M	Bhan	1
205	15.0	M	Bhan	12
206	14.0	M	Bhan	17
207	12.0	M	Bhan	0
208	13.0	M	Bhan	0
209	10.0	M	Bhan	1
210	14.0	M	Bhan	4
211	2.0	M	Bhan	0
212	2.0	M	Bhan	4
213	16.0	F	Bhan	4
214	1.0	F	Bhan	18
215	7.0	M	Bhan	0
216	5.0	M	Bhan	5
217	1.0	M	Bhan	0
218	9.0	F	Bhan	0
219	12.0	M	Bhan	52
220	7.0	M	Bhan	0
221	1.0	M	Bhan	23
222	7.0	F	Jhum	95
223	6.0	M	Jhum	4
224	2.5	M	Jhum	7
225	7.0	M	Jhum	4
226	8.0	M	Jhum	16
227	10.0	M	Jhum	0
228	6.0	F	Jhum	0
229	8.0	M	Jhum	3
230	9.0	M	Jhum	0
231	12.0	M	Jhum	1
232	7.0	M	Jhum	0
233	10.0	M	Jhum	0
234	7.0	M	Jhum	0
235	9.0	M	Jhum	1
236	6.0	M	Jhum	9
237	1.0	M	Jhum	1
238	0.4	M	Jhum	0
239	5.0	M	Jhum	5
240	5.0	M	Jhum	7
241	5.0	M	Jhum	4
242	2.0	F	Jhum	5
243	4.0	F	Jhum	0
244	8.0	F	Jhum	18
245	1.0	M	Jhum	4
246	10.0	F	Jhum	3
247	3.0	M	Jhum	11
248	6.0	F	Jhum	5
249	3.0	F	Jhum	1
250	1.0	M	Jhum	11
251	8.0	F	Jhum	0
252	9.0	M	Jhum	0
253	8.0	M	Jhum	0
254	7.0	F	Jhum	0
255	6.0	M	Jhum	0
256	7.0	M	Jhum	0
257	6.0	M	Jhum	4
258	5.0	M	Jhum	0
259	16.0	F	Jhum	0
260	7.0	F	Jhum	0
261	3.0	F	Jhum	8
262	2.0	M	Jhum	0
263	2.0	M	Jhum	0
264	1.0	M	Jhum	0
265	3.0	M	Jhum	0
266	12.0	F	Jhum	0
267	3.0	M	Jhum	5
268	12.0	F	Jhum	3
269	9.0	F	Jhum	0
270	2.0	F	Jhum	0
271	2.0	F	Jhum	8
272	14.0	F	Jhum	4
273	4.0	M	Jhum	0
274	5.0	M	Jhum	0
275	2.0	M	Jhum	0
276	3.0	F	Jhum	0
277	4.0	M	Jhum	1
278	3.0	F	Jhum	7
279	12.0	M	Jhum	3
280	7.0	M	Jhum	0
281	6.0	M	Jhum	1
282	15.0	M	Jhum	4
283	12.0	M	Jhum	5
284	6.0	F	Jhum	0
285	10.0	M	Jhum	0
286	3.0	M	Jhum	0
287	5.0	M	Jhum	0
288	10.0	F	Jhum	0
289	3.0	M	Jhum	0
290	6.0	F	Jhum	13
291	3.0	M	Jhum	5
292	1.5	F	Jhum	0
293	1.5	F	Jhum	0
294	6.0	M	Jhum	0
295	12.0	M	Jhum	1
296	8.0	M	Jhum	0
297	7.0	M	Jhum	23
298	8.0	M	Jhum	0
299	6.0	M	Jhum	1
300	4.0	M	Jhum	0
301	1.0	F	Jhum	0
302	1.0	M	Jhum	0
303	12.0	F	Jhum	7
304	10.0	F	Jhum	0
305	1.0	F	Jhum	0
306	10.0	F	Jhum	1
307	11.0	F	Jhum	0
308	10.0	F	Jhum	8
309	1.5	M	Jhum	3
310	5.0	F	Jhum	5
311	8.0	M	Jhum	0
312	9.0	M	Jhum	0
313	12.0	M	Jhum	3
314	13.0	M	Jhum	1
315	12.0	M	Jhum	0
316	14.0	M	Jhum	0
317	7.0	F	Leta	26
318	6.0	M	Leta	5
319	8.0	M	Leta	11
320	8.0	M	Leta	8
321	5.0	M	Leta	0
322	5.0	F	Leta	3
323	2.0	F	Leta	10
324	2.0	F	Leta	22
325	1.5	M	Leta	18
326	6.0	M	Leta	12
327	6.0	M	Leta	12
328	10.0	M	Leta	18
329	10.0	M	Leta	14
330	10.0	M	Leta	18
331	6.0	F	Leta	3
332	11.0	M	Leta	0
333	11.0	M	Leta	0
334	1.5	M	Leta	1
335	1.0	M	Leta	1
336	11.0	F	Leta	7
337	7.0	M	Leta	4
338	7.0	M	Leta	17
339	1.5	F	Leta	5
340	8.0	M	Leta	13
341	8.0	M	Leta	3
342	6.0	M	Leta	0
343	6.0	M	Leta	0
344	1.5	F	Leta	3
345	7.0	F	Leta	17
346	1.0	M	Leta	5
347	13.0	M	Leta	1
348	9.0	M	Leta	1
349	6.0	F	Leta	6
350	1.0	M	Leta	8
351	7.0	F	Leta	16
352	0.3	M	Leta	3
353	11.0	M	Leta	1
354	10.0	M	Leta	3
355	12.0	F	Leta	13
356	7.0	M	Leta	129
357	7.0	M	Leta	14
358	1.0	M	Leta	4
359	8.0	F	Leta	1
360	8.0	M	Leta	7
361	6.0	M	Leta	3
362	4.0	F	Leta	7
363	0.3	M	Leta	0
364	6.0	F	Leta	91
365	7.0	F	Leta	0
366	10.0	F	Leta	0
367	10.0	M	Leta	1
368	12.0	M	Leta	0
369	10.0	M	Leta	4
370	14.0	M	Leta	12
371	10.0	F	Leta	1
372	3.0	M	Leta	1
373	6.0	F	Leta	0
374	6.0	M	Leta	0
375	6.0	M	Leta	4
376	8.0	M	Leta	3
377	7.0	M	Leta	0
378	3.0	M	Leta	0
379	1.5	M	Leta	0
380	8.0	M	Leta	0
381	6.0	M	Leta	7
382	7.0	F	Leta	13
383	14.0	M	Leta	4
384	12.0	M	Leta	8
385	6.0	F	Leta	7
386	4.0	F	Leta	1
387	4.0	F	Leta	4
388	4.0	F	Leta	0
389	4.0	F	Leta	1
390	8.0	M	Leta	3
391	8.0	M	Leta	3
392	10.0	F	Leta	13
393	6.0	F	Leta	0
394	1.5	F	Leta	9
395	10.0	F	Leta	4
396	1.5	M	Leta	12
397	2.0	M	Leta	1
398	6.0	F	Leta	4
399	8.0	F	Leta	14
400	7.0	M	Leta	31
401	7.0	M	Leta	42
402	6.0	M	Leta	25
403	6.0	M	Leta	4
404	5.0	F	Leta	25
405	5.0	F	Leta	9
406	5.0	F	Leta	4
407	4.0	M	Leta	13
408	12.0	M	Leta	5
409	8.0	M	Leta	5
410	8.0	F	Leta	96
411	11.0	M	Leta	0
412	14.0	F	Leta	0
413	3.0	F	Leta	0
414	0.5	M	Leta	0
415	3.0	F	Leta	3
416	10.0	M	Leta	0
417	5.0	F	Leta	13
418	5.0	M	Leta	8
419	7.0	F	Leta	7
420	0.4	F	Leta	0
421	6.0	F	Leta	7
422	2.0	F	Leta	5
423	1.5	F	Leta	0
424	6.0	F	Leta	5

No = Animal number; Age = age of animal in years; Area = Village Development Committee: Tara = Tarahara, Bhan = Bhantabari, Jhum = Jhumka; Leta = Letang; Sys = management system, S = stall fed, G = out grazed, EPG = egg per gram of faeces; M = male, F = female.

Appendix Table 4.4.3
Farm/Flock's Cross-sectional Survey (January-February 1991)
Buffaloes in the hills

No	Age	Sex	Alti	Sys	EPG	No	Age	Sex	Alti	Sys	EPG	No	Age	Sex	Alti	Sys	EPG	No	Age	Sex	Alti	Sys	EPG
1	10.0	F	1500	S	0	86	10.0	F	1400	S	70	171	9.0	F	1750	S	0	256	6.0	F	1700	G	0
2	4.0	F	1500	S	1	87	0.5	F	1400	S	0	172	9.0	F	1750	S	0	257	7.0	F	1750	G	0
3	0.3	F	1500	S	0	88	10.0	F	1400	S	7	173	11.0	F	1750	S	26	258	4.0	F	1750	G	0
4	4.0	F	1500	S	5	89	0.6	M	1400	S	0	174	2.0	M	1750	S	0	259	6.0	F	1750	G	9
5	2.0	F	1500	S	0	90	11.0	F	1400	S	7	175	8.0	F	1700	S	3	260	8.0	F	1750	G	1
6	1.0	F	1500	S	0	91	3.0	F	1400	S	32	176	12.0	F	1700	G	7	261	5.0	F	1750	G	0
7	2.0	F	1500	S	68	92	11.0	F	1400	S	0	177	12.0	F	1700	G	92	262	9.0	F	1825	G	0
8	9.0	F	1500	S	1	93	0.6	F	1400	S	0	178	2.0	M	1700	G	114	263	3.0	M	1825	G	0
9	7.0	F	1500	S	0	94	8.0	F	1400	S	1	179	3.0	F	1750	G	73	264	9.0	F	1825	G	36
10	8.0	F	1500	S	32	95	0.7	F	1400	S	1	180	4.0	F	1750	G	30	265	7.0	F	1800	G	12
11	11.0	F	1500	S	217	96	5.0	F	1400	S	12	181	9.0	F	1750	G	1	266	3.0	F	1800	G	7
12	0.6	M	1500	S	0	97	10.0	F	1400	G	0	182	3.0	F	1750	G	5	267	6.0	F	1825	G	8
13	2.6	F	1500	S	118	98	9.0	F	1400	S	7	183	9.0	F	1700	G	72	268	10.0	F	1850	G	3
14	8.0	F	1500	S	79	99	3.0	F	1400	S	8	184	12.0	F	1700	G	0	269	3.0	F	1850	G	0
15	1.5	M	1500	S	32	100	8.0	F	1400	S	1	185	7.0	F	1700	G	0	270	3.0	F	1850	G	0
16	9.0	F	1500	S	24	101	1.5	M	1400	S	1	186	5.0	F	1700	G	27	271	8.0	F	1850	G	0
17	4.0	F	1500	S	4	102	12.0	F	1400	S	1	187	7.0	F	1725	G	30	272	6.0	F	1850	G	0
18	3.0	F	1500	G	163	103	1.3	F	1400	S	0	188	4.0	F	1725	G	77	273	3.0	M	1850	G	0
19	0.6	F	1500	G	0	104	7.0	F	1400	S	4	189	2.0	F	1725	G	0	274	6.0	F	1850	G	0
20	6.0	F	1350	S	0	105	9.0	F	1400	S	1	190	8.0	F	1725	G	104	275	6.0	F	1850	G	0
21	9.0	F	1350	S	8	106	3.0	F	1400	S	62	191	12.0	F	1725	G	24	276	3.0	F	1850	G	0
22	2.0	F	1400	G	0	107	3.0	F	1400	S	3	192	9.0	F	1725	G	30	277	4.0	F	1900	G	0
23	6.0	F	1400	G	0	108	3.0	F	1400	S	4	193	4.0	F	1725	G	24	278	3.0	F	1900	G	0
24	9.0	F	1350	S	0	109	8.0	F	1400	S	4	194	12.0	F	1750	G	31	279	8.0	F	1900	G	32
25	0.5	M	1350	S	0	110	4.0	F	1400	S	1	195	3.0	F	1750	G	0	280	5.0	F	1900	G	0
26	4.0	F	1350	G	48	111	1.5	F	1400	S	1	196	2.0	F	1750	G	21	281	15.0	F	1900	G	0
27	7.0	F	1400	S	4	112	11.0	F	1400	G	4	197	5.0	F	1750	G	77	282	6.0	F	1900	G	0
28	8.0	F	1400	S	0	113	7.0	F	1400	G	68	198	7.0	F	1700	G	12	283	5.0	F	1900	G	0
29	1.5	F	1400	S	0	114	7.0	F	1400	S	0	199	1.6	M	1700	G	0	284	3.0	F	1900	G	0
30	9.0	F	1400	G	12	115	12.0	F	1400	S	0	200	1.6	F	1700	G	0	285	3.0	F	1900	G	0
31	4.0	F	1400	G	0	116	3.0	F	1400	S	0	201	9.0	F	1700	G	4	286	7.0	F	1900	G	0
32	4.0	F	1400	S	0	117	0.5	M	1400	S	0	202	10.0	F	1700	G	0	287	7.0	F	1900	G	0
33	3.0	M	1400	S	9	118	7.0	F	1400	S	0	203	11.0	F	1700	G	0	288	9.0	F	1800	G	0
34	6.0	F	1400	S	28	119	9.0	F	1400	S	0	204	1.0	M	1700	G	0	289	3.0	F	1800	G	0
35	2.0	M	1400	S	5	120	1.0	M	1400	S	11	205	1.6	F	1700	G	0	290	4.0	F	1800	G	0
36	8.0	F	1400	S	0	121	10.0	F	1400	S	21	206	10.0	F	1700	G	22	291	13.0	F	1800	G	0
37	1.5	M	1400	S	0	122	2.0	F	1400	S	3	207	11.0	F	1700	G	0	292	4.0	M	1800	G	0
38	3.0	F	1400	S	4	123	6.0	F	1400	S	0	208	5.0	F	1700	G	51	293	15.0	F	1800	G	0
39	4.0	F	1400	S	0	124	4.0	F	1400	S	0	209	7.0	F	1700	G	1	294	12.0	F	1800	G	0
40	8.0	F	1400	S	38	125	11.0	F	1400	S	0	210	9.0	F	1800	G	0	295	4.0	M	1800	G	0
41	0.5	M	1400	S	5	126	2.0	M	1400	S	0	211	4.0	F	1800	G	0	296	3.0	F	1800	G	0
42	9.0	F	1400	S	73	127	3.0	M	1400	S	0	212	11.0	F	1800	G	0	297	6.0	F	1800	G	0
43	6.0	F	1400	S	0	128	1.0	F	1400	S	0	213	2.0	F	1800	G	0	298	8.0	F	1800	G	0
44	9.0	F	1400	S	0	129	10.0	F	1400	S	0	214	9.0	F	1800	G	34	299	3.0	M	1800	G	0
45	0.5	F	1400	S	0	130	0.7	M	1400	S	0	215	2.0	F	1800	G	0	300	12.0	F	1800	G	0
46	2.0	F	1400	S	0	131	10.0	F	1400	S	0	216	3.0	F	1750	G	0	301	6.0	F	1800	G	0
47	9.0	F	1400	S	0	132	11.0	F	1400	S	0	217	6.0	F	1750	G	0	302	12.0	F	1800	G	0
48	2.5	F	1400	S	0	133	8.0	F	1400	S	0	218	3.0	F	1750	G	0	303	4.0	M	1800	G	0
49	3.0	F	1400	S	0	134	2.5	M	1400	S	0	219	7.0	F	1750	G	0	304	9.0	F	1800	G	0
50	7.0	F	1400	S	0	135	1.0	M	1400	S	0	220	4.0	F	1750	G	0	305	2.0	M	1775	G	0
51	9.0	M	1400	S	28	136	2.0	M	1400	S	0	221	8.0	F	1750	G	0	306	10.0	F	1775	G	0
52	1.5	F	1400	S	0	137	3.0	F	1400	S	0	222	8.0	F	1750	G	3	307	6.0	F	1775	G	0
53	0.7	F	1400	S	0	138	11.0	F	1400	S	3	223	3.0	F	1750	G	0	308	5.0	F	1775	G	0
54	9.0	F	1400	S	1	139	8.0	F	1400	S	0	224	9.0	F	1750	G	0	309	7.0	F	1775	G	0
55	0.7	M	1400	S	0	140	5.0	F	1400	S	1	225	2.0	F	1750	G	0	310	4.0	M	1775	G	0
56	9.0	F	1400	S	0	141	4.0	M	1400	S	1	226	3.0	F	1750	G	0	311	6.0	F	1775	G	0
57	2.0	F	1400	G	9	142	2.0	F	1400	S	0	227	11.0	F	1750	G	3	312	5.0	F	1775	G	0
58	8.0	F	1400	G	9	143	7.0	F	630	S	8	228	2.0	M	1750	G	0	313	9.0	F	1775	G	0
59	10.0	F	1400	G	0	144	6.0	F	630	S	0	229	10.0	F	1750	G	0	314	4.0	M	1775	G	0
60	5.0	F	1400	G	0	145	3.0	M	600	S	0	230	8.0	F	1750	G	0	315	2.0	F	2000	G	0
61	14.0	F	1400	S	1	146	2.5	F	600	S	85	231	5.0	F	1750	G	0	316	7.0	F	2000	G	0
62	7.0	F	1400	S	1	147	0.6	F	600	S	0	232	9.0	F	1750	G	0	317	6.0	F	2000	G	1
63	0.5	F	1400	S	0	148	8.0	F	600	S	10	233	7.0	F	1750	G	5	318	8.0	F	2400	G	0
64	11.0	F	1400	S	7	149	9.0	F	600	S	0	234	9.0	F	1750	G	3	319	7.0	F	2400	G	0
65	13.0	F	1400	S	0	150	1.5	M	600	S	0	235	2.0	F	1750	G	28	320	10.0	F	2400	G	0
66	2.0	F	1400	S	0	151	3.0	F	600	S	0	236	9.0	F	1750	G	10	321	7.0	F	2400	G	0
67	12.0	F	1400	S	4	152	8.0	F	600	S	1	237	8.0	F	1750	G	3	322	4.0	F	2400	G	0
68	12.0	F	1400	S	0	153	9.0	F	500	S	0	238	3.0	F	1750	G	1	323	4.0	F	2400	G	0
69	2.0	F	1400	S	4	154	5.0	F	500	S	1	239	8.0	F	1750	G	0	324	5.0	F	2400	G	0
70	12.0	F	1400	S	85	155	0.6	F	500	S	0	240	4.0	F	1750	G	0	325	6.0	F	2400	G	0
71	6.0	F	1400	S	90	156	11.0	F	450	S	9	241	9.0	F	1750	G	0	326	6.0	F	2400	G	0
72	4.0	F	1400	S	10	157	3.0	F	450	S	26	242	10.0	F	1750	G	8	327	8.0	F	2400	G	0
73	2.0	F	1400	S	254	158	4.0	M	450	S	12	243	8.0	F	1750	G	1	328	6.0	F	2100	G	0
74	11.0	F	1400	S	26	159	5.0	F	450	S	8	244	3.0	M	1750	G	0	329	7.0	F	2100	G	0
75	7.0	F	1400	S	0	160	14.0	F	350	S	7	245	5.0	F	1750	G	1	330	4.0	M	2100	G	0
76	3.0	F	1400	S	0	161	1.5	F	350	S	4	246	6.0	F	1750	G	1	331	8.0	F	2150	G	0
77	1.5	M	1400	S	0	162	4.0	F	400	S	7	247	2.0	F	1750	G	0	332	7.0	F	2150	G	1
78	6.0	F	1400	G	1	163	3.0	F	400	S	4	248	7.0	F	1750	G	0	333	2.0	M	2150	G	0
79	4.0	F	1400	S	108	164	2.0	F	650	S	0	249	8.0	F	1750	G	0	334	2.0	F	2150	G	0
80	12.0	F	1400	S	0	165	1.0	F	650	S	0	250	5.0	M	1750	G	0	335	8.0	F	2900	G	88
81	4.0	F	1400	S	0	166	7.0	F	650	S	0	251	6.0	F	1700	G	0	336	6.0	F	2900	G	16
82	4.0	F	1400	S	19	167	11.0	F	650	S	0	252	2.0	M	1700	G	1	337	5.0	F	2900	G	25
83	2.0	M	1400	S	0	168	1.0	M	650	S	0	253	9.0	F	1700	G	0						
84	9.0	F	1400	S	1	169	11.0	F	650	S	0	254	10.0	F	1700	G	0						
85	4.0	F	1400	S	87	170	2.0	F	650	S	0	255	7.0	F	1700	G	0						

No =Animal number; Age =age of animal in years; Alti =elevation (in metre) of the farm; Sys =management system, S =stallfed, G =outgrazed;
EPG =eggs per gram of faeces. M =male, F =female.

Appendix Table 4.4.4
Farm/Flock's Cross-sectional Survey (March 1991)
Buffaloes in the Terai

No	Age	Sex	Area	EPG
1	9.0	F	Tara	27
2	11.0	F	Tara	151
3	1.0	M	Tara	2.6
4	11.0	F	Tara	2.6
5	1.6	F	Tara	0
6	10.0	F	Tara	12
7	0.5	M	Tara	0
8	2.0	F	Tara	9.1
9	7.0	F	Tara	17
10	2.0	F	Tara	1.3
11	1.0	M	Tara	0
12	8.0	F	Tara	38
13	1.0	M	Tara	0
14	7.0	F	Tara	0
15	8.0	F	Tara	39
16	13.0	F	Tara	35
17	0.7	F	Tara	0
18	8.0	F	Tara	3.9
19	10.0	F	Tara	2.6
20	2.0	M	Tara	3.9
21	0.9	M	Tara	0
22	2.0	F	Tara	0
23	10.0	F	Tara	0
24	10.0	F	Tara	2.6
25	2.0	F	Tara	0
26	12.0	F	Tara	7.8
27	1.0	F	Tara	0
28	14.0	F	Tara	100
29	9.0	F	Tara	38
30	6.0	F	Tara	28
31	8.0	F	Tara	14
32	12.0	F	Tara	1.3
33	9.0	F	Tara	3.9
34	7.0	F	Tara	3.9
35	3.0	F	Tara	0
36	6.0	F	Tara	1.3
37	6.0	F	Tara	0
38	7.0	F	Tara	0
39	4.0	F	Tara	0
40	8.0	F	Tara	0
41	9.0	F	Tara	0
42	5.0	F	Tara	0
43	0.6	F	Tara	0
44	0.8	M	Tara	0
45	0.8	F	Tara	0
46	10.0	F	Tara	0
47	9.0	F	Tara	9.1
48	11.0	F	Tara	13
49	0.8	F	Tara	3.9
50	7.0	F	Tara	3.9
51	0.6	M	Tara	0
52	5.0	F	Tara	3.9
53	14.0	F	Tara	1.3
54	6.0	F	Tara	17
55	8.0	F	Tara	0
56	0.6	F	Tara	1.3
57	9.0	F	Tara	42
58	9.0	F	Tara	0
59	10.0	F	Tara	1.3
60	2.0	F	Tara	0
61	14.0	F	Tara	0
62	1.0	M	Tara	0
63	12.0	F	Tara	1.3
64	12.0	F	Tara	108
65	10.0	F	Tara	42
66	9.0	F	Tara	1.5
67	7.0	F	Tara	0
68	3.0	F	Tara	0
69	3.0	M	Tara	0
70	12.0	F	Tara	0
71	10.0	F	Tara	30
72	11.0	M	Tara	53
73	8.0	F	Tara	23
74	2.0	F	Tara	1.5
75	7.0	F	Tara	3
76	0.8	F	Tara	0
77	12.0	F	Tara	3
78	8.0	F	Tara	18
79	14.0	F	Tara	3
80	2.0	F	Tara	0
81	14.0	F	Tara	0
82	8.0	F	Tara	3
83	10.0	F	Tara	1.5
84	7.0	F	Tara	27
85	6.0	F	Tara	1.5
86	1.0	F	Tara	0
87	12.0	F	Tara	0
88	9.0	F	Tara	0
89	8.0	F	Tara	0
90	1.0	F	Tara	0
91	12.0	F	Tara	17
92	9.0	F	Tara	18
93	10.0	F	Tara	0
94	9.0	F	Tara	0
95	0.3	F	Tara	0
96	0.7	M	Tara	0
97	8.0	F	Tara	6.1
98	9.0	F	Tara	55
99	0.6	F	Tara	0
100	9.0	F	Tara	27
101	2.6	M	Tara	0
102	8.0	F	Tara	0
103	12.0	F	Tara	0
104	3.0	M	Tara	107
105	2.8	M	Tara	6.1
106	2.9	M	Tara	1.5
107	2.8	M	Tara	7.6
108	2.9	M	Tara	9.1
109	2.8	M	Tara	0
110	12.0	M	Tara	1.5
111	2.9	M	Tara	1.5
112	5.8	M	Tara	0
113	11.0	M	Tara	1.5
114	4.6	M	Tara	0
115	1.7	M	Tara	0
116	5.9	F	Tara	0
117	8.3	F	Tara	0
118	12.0	F	Tara	0
119	6.0	F	Tara	0
120	7.0	F	Tara	0
121	13.0	F	Tara	0
122	9.0	F	Tara	0
123	7.0	F	Tara	1.5
124	9.0	F	Tara	0
125	13.0	F	Tara	0
126	6.0	F	Tara	0
127	8.0	F	Tara	0
128	9.0	F	Tara	0
129	6.0	F	Tara	0
130	3.9	M	Tara	0
131	13.0	M	Tara	0
132	13.0	M	Tara	7.6
133	13.0	M	Tara	4.6
134	14.0	M	Tara	1.5
135	3.0	M	Tara	0
136	3.0	M	Tara	0
137	3.0	M	Tara	0
138	3.0	M	Tara	0
139	2.6	M	Bhan	0
140	3.0	M	Bhan	1.5
141	7.0	M	Bhan	0
142	6.0	M	Bhan	0
143	0.4	M	Bhan	0
144	0.8	F	Bhan	0
145	0.9	F	Bhan	0
146	0.9	F	Bhan	0
147	0.6	F	Bhan	0
148	0.8	M	Bhan	0
149	11.0	F	Bhan	0
150	3.0	F	Bhan	0
151	9.0	F	Bhan	0
152	4.0	F	Bhan	0
153	3.0	F	Bhan	0
154	2.0	F	Bhan	0
155	15.0	F	Bhan	1.5
156	18.0	F	Bhan	0
157	15.0	F	Bhan	0
158	4.0	M	Bhan	0
159	3.0	M	Bhan	1.5
160	10.0	F	Bhan	0
161	9.0	F	Bhan	0
162	15.0	F	Bhan	0
163	18.0	F	Bhan	3
164	9.0	F	Bhan	15
165	13.0	F	Bhan	0
166	21.0	M	Bhan	0
167	5.0	M	Bhan	0
168	6.0	M	Bhan	0
169	4.0	M	Bhan	0
170	4.0	F	Bhan	0
171	8.0	F	Bhan	0
172	16.0	F	Bhan	0
173	6.0	F	Bhan	0
174	1.0	F	Bhan	0
175	10.0	F	Bhan	0
176	6.0	F	Bhan	0
177	0.5	F	Bhan	0
178	3.0	F	Bhan	0
179	9.0	F	Bhan	4.6
180	16.0	F	Bhan	0
181	1.5	M	Bhan	0
182	6.0	F	Bhan	0
183	14.0	F	Bhan	0
184	0.7	F	Bhan	0
185	7.0	F	Bhan	0
186	8.0	F	Bhan	0
187	12.0	M	Bhan	15
188	1.0	F	Bhan	0
189	9.0	F	Bhan	0
190	2.0	F	Bhan	1.5
191	1.0	F	Bhan	0
192	3.0	F	Bhan	6.1
193	14.0	F	Bhan	4.6
194	0.6	F	Bhan	1.5
195	8.0	F	Bhan	0
196	14.0	F	Bhan	0
197	4.0	F	Bhan	0
198	9.0	F	Bhan	0
199	0.7	M	Bhan	3
200	15.0	F	Bhan	0
201	5.5	F	Bhan	11
202	1.0	F	Bhan	0
203	8.0	F	Bhan	0
204	11.0	F	Bhan	0
205	9.0	F	Leta	7.6
206	2.0	M	Leta	41
207	10.0	F	Leta	4.6
208	0.7	F	Leta	11
209	1.4	F	Leta	4.6
210	1.0	F	Leta	9.1
211	1.0	M	Leta	1.5
212	11.0	F	Leta	4.6
213	0.7	M	Leta	4.6
214	5.0	F	Leta	1.5
215	7.0	F	Leta	0
216	6.0	F	Leta	15
217	2.0	F	Leta	0
218	1.0	M	Leta	0
219	6.0	F	Leta	0
220	8.0	F	Leta	0
221	7.0	F	Leta	91
222	1.4	M	Leta	0
223	11.0	F	Leta	4.6
224	1.5	M	Leta	0
225	9.0	F	Leta	1.5
226	5.0	M	Leta	3
227	8.0	F	Leta	4.6
228	10.0	F	Leta	1.5
229	11.0	F	Leta	0
230	1.4	M	Leta	0
231	14.0	F	Leta	3
232	3.0	M	Leta	0
233	6.0	F	Leta	0
234	0.9	M	Leta	0
235	8.0	F	Leta	0
236	0.9	F	Leta	0
237	8.0	F	Leta	0
238	0.9	M	Leta	0
239	11.0	F	Leta	0
240	0.6	M	Leta	0
241	2.0	F	Leta	0
242	14.0	F	Leta	0
243	7.0	F	Leta	0
244	0.6	F	Leta	0
245	7.0	F	Leta	0
246	0.5	M	Leta	0
247	6.0	F	Leta	0
248	7.0	F	Leta	0
249	1.4	M	Leta	0
250	1.0	F	Leta	0
251	0.4	M	Leta	0
252	10.0	F	Leta	0
253	6.0	F	Leta	0
254	0.5	M	Leta	0
255	9.0	F	Leta	0
256	6.0	F	Leta	47
257	12.0	F	Leta	1.5
258	9.0	F	Leta	4.6
259	0.6	M	Leta	0
260	8.0	F	Leta	2.7
261	10.0	F	Leta	6.1
262	0.5	M	Leta	4.6
263	7.0	F	Leta	50
264	12.0	F	Leta	3
265	8.0	F	Leta	0
266	4.0	F	Leta	0
267	6.0	F	Leta	0
268	10.0	F	Leta	0
269	9.0	F	Leta	1.5
270	8.0	F	Leta	0
271	1.6	M	Leta	3
272	7.0	F	Leta	3
273	4.0	F	Leta	4.6
274	5.0	F	Leta	6.1
275	7.0	F	Leta	4.6
276	7.0	F	Leta	0
277	0.9	M	Leta	0
278	8.0	F	Leta	0
279	1.0	F	Leta	0
280	9.0	F	Leta	0
281	0.7	F	Leta	0
282	10.0	F	Leta	0
283	2.0	F	Leta	0
284	12.0	F	Leta	1.5
285	7.0	F	Leta	3
286	5.0	F	Leta	0
287	8.0	F	Leta	12
288	12.0	F	Leta	46
289	7.0	F	Leta	3
290	8.0	F	Leta	1.5
291	8.0	F	Leta	4.6
292	8.0	F	Leta	6.1
293	6.0	F	Leta	18
294	6.0	F	Leta	13
295	8.0	F	Leta	9.1
296	0.7	F	Leta	0
297	6.0	F	Leta	0
298	8.0	F	Leta	0

No = Animal number; Age = age of animal in years; Area = Village Development Committee: Tara = Tarahara, Bhan = Bhantabari, Jhum = Jhumka, Leta = Letang; Sys = management system, S = stall fed, G = out grazed; EPG = egg per gram of faeces; M = male, F= female.

Appendix Table 4.4.5
Farm/Flock's Cross-sectional Survey (January-February 1991)
Goats in the hills

No	Age	Sex	Alti	EPG
1	7.0	F	1500	0
2	4.0	F	1500	0
3	2.0	F	1500	0
4	0.8	M	1500	0
5	1.0	M	1500	0
6	0.6	M	1500	0
7	6.0	F	1500	0
8	1.5	F	1500	0
9	1.5	F	1475	8
10	2.0	F	1475	0
11	1.0	M	1475	0
12	2.0	F	1475	0
13	0.7	M	1475	0
14	5.0	F	1475	8
15	1.5	F	1475	0
16	3.0	F	1400	0
17	10.0	F	1400	29
18	2.0	F	1400	105
19	1.0	F	1400	0
20	3.0	M	1450	0
21	2.0	M	1450	0
22	5.0	F	1450	10
23	5.0	F	1450	0
24	2.0	F	1450	0
25	6.0	F	1450	0
26	4.0	F	1450	0
27	8.0	F	1450	0
28	0.5	F	1450	0
29	6.0	F	1450	0
30	2.0	F	1450	1
31	2.0	F	1425	7
32	7.0	F	1425	0
33	1.5	M	1400	3
34	2.0	M	1400	0
35	3.0	M	1400	1
36	2.0	F	1400	1
37	3.0	F	1400	0
38	4.0	F	1400	0
39	2.0	M	1400	0
40	8.0	F	1400	1
41	7.0	F	1400	25
42	1.0	F	1400	0
43	3.0	F	1400	4
44	4.0	F	1375	8
45	5.0	F	1375	7
46	3.0	F	1375	0
47	8.0	F	1375	0
48	4.0	F	1375	0
49	5.0	F	1375	0
50	3.0	F	1375	0
51	5.0	F	1375	0
52	4.0	F	1350	0
53	4.0	F	1350	0
54	5.0	F	1350	0
55	3.0	F	1350	1
56	1.0	F	1350	0
57	2.0	F	1350	68
58	4.0	F	1350	27
59	5.0	F	1350	114
60	2.0	M	1350	1
61	3.0	F	1350	9
62	3.0	M	1350	10
63	6.0	F	1350	124
64	2.0	M	1350	5
65	1.5	M	1350	0
66	1.0	F	1350	0
67	6.0	F	1350	3
68	8.0	F	1350	12
69	7.0	F	1375	0
70	6.0	F	1375	3
71	5.0	F	1375	0
72	2.0	M	1375	0
73	5.0	F	1375	10
74	3.0	F	1375	5
75	4.0	F	1375	3
76	5.0	F	1350	8
77	4.0	F	1350	72
78	4.0	F	1350	27
79	1.0	M	1350	0
80	4.0	F	1350	30
81	5.0	F	1350	25
82	3.0	F	1350	0
83	1.0	M	1350	0
84	3.0	F	1375	5
85	6.0	F	1375	0
86	1.0	M	1375	0
87	2.0	F	1375	0
88	1.0	M	1375	0
89	4.0	F	1375	0
90	1.0	M	1375	0
91	4.0	F	1400	0
92	4.0	F	1400	4
93	4.0	F	1300	0
94	5.0	F	1300	0
95	4.0	F	1300	0
96	2.0	F	1300	0
97	0.5	F	1300	0
98	9.0	F	1300	52
99	6.0	F	1300	0
100	4.0	M	1300	0
101	3.0	F	1300	8
102	1.0	M	1300	0
103	4.0	F	1300	0
104	1.0	F	1325	0
105	1.0	F	1325	0
106	6.0	F	1325	10
107	3.0	M	1325	0
108	2.0	F	1325	0
109	3.0	F	1325	0
110	8.0	F	1300	0
111	7.0	F	1300	0
112	2.0	M	1300	0
113	4.0	F	1300	0
114	2.0	F	1300	0
115	1.0	M	1300	0
116	6.0	F	1300	0
117	3.0	F	1300	0
118	4.0	F	1300	0
119	4.0	F	1325	0
120	3.0	F	1325	0
121	1.5	M	1325	0
122	2.0	F	1325	0
123	3.0	F	1325	0
124	1.0	M	1325	0
125	3.0	F	1325	3
126	1.0	F	1325	0
127	1.0	F	1325	0
128	3.0	F	1325	0
129	3.0	F	1325	0
130	9.0	F	1325	4
131	7.0	F	1200	16
132	5.0	F	1200	3
133	7.0	F	1200	14
134	4.0	M	1200	0
135	5.0	F	1200	3
136	4.0	F	1200	0
137	9.0	F	1200	0
138	1.0	M	1200	0
139	2.0	F	1200	20
140	5.0	F	1200	7
141	3.0	F	1200	18
142	3.0	F	1200	9
143	3.0	M	1200	0
144	2.0	M	1200	1
145	5.0	F	1200	0
146	4.0	F	1200	0
147	3.0	M	1200	0
148	6.0	F	1250	0
149	2.0	F	1250	0
150	3.0	F	1225	0
151	1.0	M	1225	0
152	3.0	F	1225	0
153	9.0	F	1225	0
154	3.0	F	1225	0
155	4.0	F	1225	1
156	2.0	M	1225	0
157	1.5	F	1225	0
158	2.0	M	1250	0
159	2.0	F	1250	0
160	4.0	F	1250	0
161	3.0	M	1250	0
162	1.0	M	1250	0
163	3.0	F	1250	0
164	4.0	F	1250	0
165	0.5	M	1250	0
166	2.0	M	1225	0
167	4.0	F	1225	0
168	4.0	F	1225	1
169	1.0	M	1250	0
170	4.0	F	1250	0
171	1.0	M	1250	1
172	2.0	F	1250	0
173	4.0	F	1225	0
174	1.5	M	1225	0
175	3.0	F	1225	0
176	5.0	F	1225	0
177	3.0	F	1225	0
178	2.0	F	1225	0
179	2.0	M	1300	0
180	2.0	M	1300	0
181	3.0	F	1300	0
182	2.0	F	1300	0
183	2.0	M	1300	0
184	4.0	F	1300	0
185	5.0	F	1300	0
186	6.0	F	1300	0
187	6.0	F	1300	0
188	2.0	M	1225	0
189	2.0	M	1100	0
190	3.0	M	1100	0
191	5.0	F	1100	0
192	3.0	F	1100	0
193	4.0	F	1100	0
194	2.0	F	1100	0
195	1.0	M	1100	0
196	2.0	M	1100	0
197	3.0	F	1100	0
198	4.0	F	1000	0
199	7.0	F	1000	3
200	3.0	F	1050	0
201	5.0	F	1050	0
202	4.0	F	1050	0
203	5.0	F	1050	0
204	2.0	M	1050	0
205	5.0	F	1000	0
206	4.0	F	1000	0
207	1.0	F	1000	0
208	7.0	F	1100	0
209	5.0	F	1100	0
210	9.0	F	1250	4
211	2.0	M	1250	5
212	1.0	M	1250	0
213	5.0	F	1300	4
214	4.0	F	1300	1
215	3.0	F	1300	20
216	2.0	F	1300	0
217	2.0	F	1300	0
218	1.5	F	1300	0
219	2.0	M	1250	0
220	2.0	M	1250	0
221	3.0	M	1250	0
222	2.0	F	1250	0
223	3.0	F	1250	0
224	4.0	F	1250	3
225	3.0	F	1250	0
226	6.0	F	1250	0
227	1.0	M	1250	0
228	5.0	F	1250	1
229	1.0	M	1250	0
230	2.0	M	850	0
231	3.0	F	850	0
232	2.0	F	850	0
233	2.0	M	850	0
234	5.0	F	850	0
235	0.6	M	850	0
236	0.4	M	850	0
237	4.0	F	850	0
238	1.0	M	850	0
239	1.0	M	850	0
240	4.0	F	850	0
241	5.0	F	850	1
242	1.0	F	850	0
243	5.0	F	850	0
244	3.0	F	825	0
245	2.0	F	825	0
246	3.0	F	825	0
247	4.0	F	825	0
248	1.0	F	825	0
249	4.0	F	825	0
250	2.0	F	825	0
251	1.0	M	825	0
252	0.8	F	825	0
253	1.5	M	825	0
254	1.5	F	825	0
255	5.0	F	825	0
256	4.0	F	825	1
257	5.0	F	825	1
258	2.0	F	825	0
259	6.0	F	825	0
260	1.0	F	825	0
261	1.5	M	825	0
262	2.0	F	825	0
263	0.9	F	850	5
264	0.9	F	850	0
265	5.0	F	850	0
266	0.8	M	850	0
267	4.0	F	825	0
268	5.0	F	825	0
269	0.3	F	825	0
270	1.0	F	825	0
271	1.0	M	825	0
272	1.0	M	825	0
273	4.0	F	825	0
274	1.0	F	825	9
275	4.0	F	800	0
276	4.0	F	800	0
277	1.0	M	800	0
278	2.0	M	800	0
279	5.0	F	800	0
280	1.0	M	800	0
281	1.0	F	850	0
282	1.0	M	850	0
283	4.0	F	850	0
284	2.0	M	850	0
285	1.0	M	850	0
286	1.0	M	850	0
287	2.5	M	850	0
288	1.0	F	900	0
289	0.9	F	900	0
290	4.0	F	900	0
291	3.0	F	900	0
292	1.0	F	900	0
293	1.0	F	900	0
294	3.0	F	900	0
295	1.5	M	900	0
296	2.0	F	900	0
297	1.5	M	900	0
298	2.0	M	900	0
299	4.0	F	900	0
300	1.0	M	875	0
301	1.0	F	875	0
302	0.9	M	875	0
303	2.0	M	875	3
304	3.0	M	875	0
305	4.0	F	875	0
306	0.9	M	800	0
307	2.0	F	800	0
308	3.0	F	800	0
309	4.0	F	650	0
310	4.0	F	650	0
311	1.0	F	650	0
312	3.0	F	650	0
313	5.0	F	650	0
314	3.0	F	650	0
315	2.0	M	650	0
316	4.0	F	650	0
317	3.0	F	650	0
318	4.0	F	650	13
319	3.0	F	650	0
320	3.0	F	450	3
321	7.0	F	450	0
322	5.0	F	450	3
323	1.0	M	450	29
324	2.0	M	650	10
325	1.0	M	650	3
326	4.0	F	650	0
327	3.0	F	650	0
328	4.0	F	650	0
329	5.0	F	650	0
330	7.0	F	450	0
331	5.0	F	450	0
332	4.0	F	450	0
333	2.0	M	450	0
334	3.0	F	450	0
335	1.0	M	450	5
336	7.0	F	450	8
337	5.0	F	450	0
338	2.0	F	450	0
339	2.0	F	650	0
340	2.0	M	650	0
341	5.0	F	650	0
342	0.5	F	650	0
343	0.8	M	650	0
344	3.0	F	650	0
345	8.0	F	650	0
346	4.0	F	650	0
347	5.0	F	700	0
348	1.0	F	700	0
349	5.0	F	700	0
350	1.0	F	700	3
351	3.0	F	700	0
352	4.0	F	700	5
353	5.0	F	650	0
354	2.0	F	650	0
355	8.0	F	650	0
356	3.0	F	650	0
357	1.5	M	650	0
358	1.5	M	650	0
359	2.0	M	650	0
360	1.5	M	650	10
361	1.0	M	650	0
362	0.5	F	650	0
363	2.0	F	650	0
364	5.0	F	650	0
365	4.0	F	650	0
366	3.0	F	650	10
367	1.0	M	630	5
368	2.0	M	650	0
369	3.0	F	650	0
370	4.0	F	650	0
371	2.0	M	650	0
372	3.0	F	650	0
373	4.0	F	650	0
374	3.0	F	650	0
375	5.0	F	650	0
376	6.0	F	650	0
377	1.0	M	630	0
378	1.0	F	630	0
379	4.0	F	630	0
380	2.0	F	630	0
381	2.0	F	630	0
382	3.0	F	630	0
383	0.5	M	600	0
384	4.0	F	600	0
385	1.0	M	600	0
386	4.0	F	600	0
387	2.5	M	630	0
388	2.0	F	500	0
389	4.0	F	550	0
390	0.5	F	550	0
391	2.0	F	550	0
392	2.0	F	575	0
393	1.0	F	575	0
394	2.0	M	575	0
395	1.0	M	575	0
396	2.0	F	525	0
397	1.0	F	525	0
398	4.0	F	525	0
399	3.0	F	525	0
400	3.0	F	525	0
401	3.0	F	400	3
402	4.0	F	400	0
403	4.0	F	400	0
404	1.0	M	400	0
405	3.0	F	400	3
406	2.0	F	400	8
407	3.0	F	400	0
408	3.0	F	400	5
409	2.0	F	350	0
410	3.0	F	350	0
411	2.0	M	350	0
412	3.0	F	350	0
413	2.0	F	350	0
414	5.0	F	350	0
415	3.0	F	350	0
416	3.0	F	350	0
417	12.0	F	400	0
418	3.0	F	400	0
419	0.5	M	400	0
420	1.0	F	400	0
421	1.0	M	550	0
422	4.0	F	550	0
423	3.0	F	550	0
424	2.0	F	550	0
425	5.0	F	550	0
426	3.0	F	550	0
427	2.0	F	550	0
428	2.0	F	600	0
429	3.0	F	600	0
430	1.0	F	600	0
431	4.0	F	600	0
432	1.0	M	600	0
433	2.0	F	600	0
434	2.0	F	600	0
435	4.0	F	600	0
436	3.0	F	1700	0
437	2.0	F	1700	0
438	1.0	M	1750	0
439	2.0	M	1750	0
440	3.0	M	1750	0
441	2.0	M	1750	0
442	2.0	M	1750	0
443	2.0	F	1750	0
444	4.0	F	1700	0
445	3.0	F	1700	0
446	4.0	F	1550	0
447	4.0	F	1550	3
448	2.0	F	1550	0
449	4.0	F	1550	3
450	1.0	M	1550	0
451	4.0	F	1550	8
452	1.0	M	1550	0
453	1.0	M	1700	0
454	4.0	F	1750	0
455	3.0	F	1750	5
456	4.0	M	1750	0
457	2.0	M	1750	0
458	4.0	F	1750	29
459	2.0	F	1750	0
460	1.0	M	1750	0
461	2.0	F	1750	0
462	3.0	F	1750	0
463	2.0	F	1750	0
464	1.0	M	1750	0
465	3.0	F	1750	0
466	4.0	M	1750	0
467	3.0	M	1750	0
468	5.0	M	1750	0
469	5.0	M	1750	0
470	5.0	F	1750	0
471	7.0	F	1750	0
472	8.0	F	1750	0
473	5.0	F	1750	0
474	12.0	F	1750	8
475	3.0	F	1750	0
476	2.0	F	1750	0
477	2.0	M	1750	0
478	4.0	F	1750	0
479	5.0	F	1750	0
480	1.0	M	1750	0
481	5.0	F	1750	0
482	5.0	F	1750	0
483	3.0	F	1750	0
484	1.0	M	1750	0
485	8.0	F	1750	0
486	8.0	M	1750	0
487	6.0	F	1750	0
488	2.0	F	1750	0
489	1.4	F	1750	0
490	7.0	F	1750	0
491	5.0	F	1750	0
492	4.0	F	1800	0
493	1.3	M	1800	0
494	4.0	F	1800	0
495	0.8	F	1800	0
496	0.8	M	1800	0
497	2.0	F	1800	0
498	1.0	F	1800	0
499	12.0	F	1800	0
500	5.0	F	1800	0
501	5.0	F	1800	0
502	2.0	F	1800	0
503	1.3	F	1800	0
504	4.0	F	1800	0
505	1.3	M	1750	0
506	3.0	F	1750	0
507	3.0	F	1750	0
508	4.0	M	1750	0
509	2.0	M	1750	0
510	1.0	M	1750	0
511	10.0	F	1750	0
512	1.3	F	1750	0
513	10.0	F	1750	0

No =Animal number; Age =age of animals in years; Alti =elevation (in metre) of the farm; Sys =management system, S =stallfed, G =outgrazed, EPG =eggs per gram of faeces, M =male, F = female.

Appendix Table 4.4.6
Cross-sectional Survey of Farm/Flocks (February 1991)
Goats in the Terai

No	Age	Sex	Area	EPG	No	Age	Sex	Area	EPG	No	Age	Sex	Area	EPG	No	Age	Sex	Area	EPG
1	1.0	M	Tara	0.0	33	1.5	F	Tara	2.6	65	1.5	M	Leta	0.0	97	3.0	F	Bhan	0.0
2	2.0	F	Tara	0.0	34	4.0	F	Tara	0.0	66	3.0	F	Leta	0.0	98	4.0	F	Bhan	0.0
3	2.0	M	Tara	0.0	35	5.0	F	Tara	3.9	67	3.0	F	Leta	0.0	99	1.0	F	Bhan	0.0
4	1.0	M	Tara	0.0	36	0.5	M	Tara	0.0	68	3.0	F	Leta	0.0	100	2.0	F	Bhan	0.0
5	1.5	M	Tara	0.0	37	0.5	F	Tara	1.3	69	1.5	F	Leta	0.0	101	3.0	F	Bhan	0.0
6	1.0	M	Tara	0.0	38	4.0	F	Leta	0.0	70	1.0	M	Leta	0.0	102	5.0	F	Bhan	0.0
7	1.0	F	Tara	0.0	39	1.5	F	Leta	0.0	71	1.5	M	Leta	0.0	103	7.0	F	Bhan	1.3
8	2.0	F	Tara	0.0	40	5.0	F	Leta	0.0	72	2.0	F	Leta	0.0	104	1.0	F	Bhan	1.3
9	3.0	F	Tara	0.0	41	1.0	M	Leta	0.0	73	4.0	F	Leta	0.0	105	2.0	F	Bhan	0.0
10	2.0	F	Tara	0.0	42	1.0	M	Leta	0.0	74	5.0	F	Leta	3.9	106	0.7	F	Bhan	0.0
11	3.0	F	Tara	0.0	43	1.0	M	Leta	0.0	75	2.0	F	Leta	0.0	107	3.0	F	Bhan	3.9
12	2.0	F	Tara	0.0	44	1.5	M	Leta	0.0	76	2.5	F	Leta	0.0	108	4.0	F	Bhan	0.0
13	4.0	F	Tara	0.0	45	1.0	M	Leta	3.9	77	1.0	F	Leta	1.3	109	1.0	F	Bhan	0.0
14	2.0	M	Tara	0.0	46	1.0	F	Leta	1.3	78	5.0	F	Leta	0.0	110	3.0	F	Bhan	0.0
15	1.0	M	Tara	0.0	47	2.0	F	Leta	0.0	79	0.5	M	Leta	0.0	111	5.0	F	Bhan	0.0
16	1.0	M	Tara	1.3	48	1.0	F	Leta	0.0	80	5.0	F	Leta	0.0	112	2.0	F	Bhan	0.0
17	3.0	F	Tara	0.0	49	1.5	F	Leta	0.0	81	4.0	F	Leta	0.0	113	3.0	F	Bhan	0.0
18	1.0	M	Tara	0.0	50	1.0	M	Leta	0.0	82	5.0	F	Leta	0.0	114	1.0	F	Bhan	0.0
19	2.0	F	Tara	0.0	51	4.0	F	Leta	0.0	83	3.0	F	Leta	0.0	115	3.0	F	Bhan	1.3
20	1.0	M	Tara	0.0	52	1.0	M	Leta	0.0	84	5.0	F	Leta	0.0	116	2.0	F	Bhan	2.6
21	2.0	F	Tara	0.0	53	0.5	M	Leta	0.0	85	7.0	F	Leta	2.6	117	5.0	F	Bhan	2.6
22	1.0	F	Tara	0.0	54	1.5	F	Leta	0.0	86	8.0	F	Leta	0.0	118	4.0	F	Bhan	2.6
23	2.0	F	Tara	0.0	55	4.0	F	Leta	0.0	87	6.0	F	Leta	0.0	119	2.0	F	Bhan	0.0
24	1.0	M	Tara	0.0	56	2.0	M	Leta	0.0	88	3.0	F	Leta	0.0	120	4.0	F	Bhan	0.0
25	2.0	M	Tara	4.5	57	1.0	M	Leta	0.0	89	1.0	F	Leta	0.0	121	0.3	F	Bhan	0.0
26	3.0	M	Tara	0.0	58	9.0	F	Leta	0.0	90	5.0	F	Leta	0.0	122	3.0	F	Bhan	0.0
27	0.5	M	Tara	0.0	59	1.0	M	Leta	0.0	91	6.0	F	Leta	0.0	123	1.5	M	Bhan	0.0
28	2.0	M	Tara	0.0	60	0.3	M	Leta	0.0	92	1.0	M	Leta	0.0	124	0.3	M	Bhan	0.0
29	2.0	F	Tara	0.0	61	0.3	M	Leta	0.0	93	5.0	M	Leta	0.0	125	4.0	M	Bhan	0.0
30	1.5	F	Tara	0.0	62	8.0	F	Leta	0.0	94	3.0	F	Leta	0.0	126	0.3	M	Bhan	0.0
31	7.0	F	Tara	0.0	63	0.5	M	Leta	0.0	95	1.4	F	Leta	0.0	127	0.3	M	Bhan	0.0
32	1.0	F	Tara	0.0	64	1.0	M	Leta	0.0	96	2.0	F	Bhan	0.0	128	5.0	F	Bhan	0.0

N0 = Animal number, Age = age of animal in year, Area = Village Development Committee: Tara = Tarahara, Leta = Letang, Bhan = Bhantabari; EPG = egg per gram of faeces; M = male, F = female.

Appendix Table 4.4.7
Farm/Flock's Cross-sectional Survey (February 1991)
Sheep in the hills

No	Age	Sex	Alti	Sys	EPG	No	Age	Sex	Alti	Sys	EPG	No	Age	Sex	Alti	Sys	EPG	No	Age	Sex	Alti	Sys	EPG
1	8.0	F	N/A	MI	0	70	3.0	F	N/A	MI	0	139	11.0	F	N/A	MI	0	208	0.3	F	N/A	MI	0
2	0.5	F	N/A	MI	1	71	5.0	F	N/A	MI	0	140	3.0	F	N/A	MI	0	209	9.0	F	N/A	MI	0
3	6.0	F	N/A	MI	0	72	4.0	F	N/A	MI	0	141	4.0	M	N/A	MI	0	210	11.0	F	N/A	MI	0
4	1.0	F	N/A	MI	0	73	7.0	F	N/A	MI	0	142	3.0	F	N/A	MI	0	211	3.0	F	N/A	MI	0
5	3.0	F	N/A	MI	0	74	5.0	F	N/A	MI	8	143	3.0	M	N/A	MI	0	212	9.0	F	N/A	MI	0
6	9.0	F	N/A	MI	0	75	9.0	F	N/A	MI	8	144	1.0	M	N/A	MI	0	213	7.0	F	N/A	MI	0
7	0.5	M	N/A	MI	0	76	3.0	F	N/A	MI	0	145	2.0	F	N/A	MI	0	214	8.0	F	N/A	MI	0
8	10.0	F	N/A	MI	0	77	6.0	F	N/A	MI	0	146	7.0	F	N/A	MI	0	215	6.0	F	N/A	MI	0
9	4.0	F	N/A	MI	0	78	1.0	F	N/A	MI	0	147	0.7	M	N/A	MI	0	216	6.0	F	N/A	MI	0
10	0.5	M	N/A	MI	0	79	2.0	F	N/A	MI	0	148	4.0	F	N/A	MI	0	217	6.0	F	N/A	MI	26
11	1.0	F	N/A	MI	0	80	2.0	F	N/A	MI	0	149	1.0	F	N/A	MI	0	218	6.0	F	N/A	MI	5
12	5.0	F	N/A	MI	0	81	3.0	F	N/A	MI	0	150	4.0	F	N/A	MI	0	219	0.3	M	N/A	MI	0
13	2.0	F	N/A	MI	0	82	3.0	F	N/A	MI	0	151	5.0	F	N/A	MI	0	220	0.3	M	N/A	MI	0
14	8.0	F	N/A	MI	0	83	5.0	F	N/A	MI	0	152	0.4	F	N/A	MI	0	221	9.0	F	N/A	MI	0
15	7.0	F	N/A	MI	0	84	8.0	F	N/A	MI	0	153	0.4	M	N/A	MI	0	222	9.0	F	N/A	MI	0
16	0.7	M	N/A	MI	0	85	9.0	F	N/A	MI	13	154	1.0	F	N/A	MI	0	223	3.0	F	N/A	MI	0
17	10.0	F	N/A	MI	0	86	8.0	F	N/A	MI	0	155	9.0	F	N/A	MI	0	224	4.0	F	N/A	MI	0
18	7.0	F	N/A	MI	3	87	8.0	F	N/A	MI	0	156	9.0	F	N/A	MI	0	225	7.0	F	N/A	MI	0
19	11.0	F	N/A	MI	0	88	8.0	F	N/A	MI	0	157	8.0	F	N/A	MI	0	226	1.0	F	N/A	MI	0
20	2.0	M	N/A	MI	0	89	1.0	F	N/A	MI	0	158	7.0	F	N/A	MI	0	227	0.3	M	N/A	MI	0
21	0.5	M	N/A	MI	0	90	7.0	F	N/A	MI	10	159	5.0	F	N/A	MI	0	228	0.3	F	N/A	MI	0
22	0.4	F	N/A	MI	0	91	3.0	F	N/A	MI	5	160	5.0	F	N/A	MI	0	229	8.0	F	N/A	MI	0
23	0.5	F	N/A	MI	0	92	2.0	F	N/A	MI	0	161	1.0	F	N/A	MI	0	230	8.0	F	N/A	MI	0
24	8.0	F	N/A	MI	0	93	2.0	F	N/A	MI	0	162	3.0	F	N/A	MI	0	231	7.0	F	N/A	MI	0
25	7.0	F	N/A	MI	0	94	3.0	F	N/A	MI	0	163	6.0	F	N/A	MI	0	232	3.0	F	N/A	MI	0
26	6.0	F	N/A	MI	0	95	1.0	F	N/A	MI	0	164	7.0	F	N/A	MI	0	233	4.0	F	N/A	MI	0
27	8.0	F	N/A	MI	1	96	6.0	F	N/A	MI	0	165	8.0	F	N/A	MI	0	234	0.7	F	N/A	MI	0
28	3.0	M	N/A	MI	3	97	7.0	F	N/A	MI	0	166	9.0	F	N/A	MI	0	235	2.0	M	1500	SE	0
29	8.0	F	N/A	MI	3	98	8.0	F	N/A	MI	0	167	10.0	F	N/A	MI	0	236	1.0	F	1500	SE	0
30	9.0	F	N/A	MI	3	99	9.0	F	N/A	MI	0	168	4.0	M	N/A	MI	0	237	3.0	F	1500	SE	0
31	0.8	F	N/A	MI	0	100	2.0	F	N/A	MI	5	169	4.0	M	N/A	MI	0	238	2.0	M	1500	SE	0
32	1.0	M	N/A	MI	0	101	10.0	F	N/A	MI	0	170	12.0	M	N/A	MI	0	239	3.0	M	1500	SE	0
33	12.0	F	N/A	MI	3	102	9.0	F	N/A	MI	0	171	0.4	F	N/A	MI	0	240	2.0	M	1500	SE	0
34	6.0	F	N/A	MI	0	103	7.0	F	N/A	MI	0	172	7.0	F	N/A	MI	0	241	3.0	F	1500	SE	0
35	8.0	F	N/A	MI	1	104	3.0	F	N/A	MI	0	173	4.0	F	N/A	MI	0	242	4.0	F	1500	SE	26
36	9.0	F	N/A	MI	3	105	2.0	F	N/A	MI	0	174	5.0	M	N/A	MI	0	243	2.0	F	1500	SE	0
37	3.0	F	N/A	MI	0	106	1.0	F	N/A	MI	0	175	5.0	F	N/A	MI	0	244	4.0	F	1500	SE	0
38	5.0	F	N/A	MI	0	107	4.0	F	N/A	MI	0	176	3.0	F	N/A	MI	0	245	3.0	F	1500	SE	0
39	4.0	F	N/A	MI	0	108	5.0	F	N/A	MI	0	177	2.0	F	N/A	MI	0	246	2.0	F	1500	SE	0
40	4.0	F	N/A	MI	0	109	4.0	F	N/A	MI	0	178	1.0	F	N/A	MI	0	247	2.0	F	1500	SE	0
41	7.0	F	N/A	MI	3	110	4.0	F	N/A	MI	0	179	4.0	F	N/A	MI	0	248	4.0	M	1500	SE	20
42	1.0	M	N/A	MI	0	111	3.0	F	N/A	MI	0	180	4.0	F	N/A	MI	0	249	2.0	M	1500	SE	0
43	3.5	M	N/A	MI	0	112	7.0	F	N/A	MI	0	181	0.5	F	N/A	MI	0	250	1.0	F	1500	SE	0
44	0.5	M	N/A	MI	0	113	7.0	F	N/A	MI	0	182	6.0	F	N/A	MI	0	251	5.0	F	1700	SE	5
45	6.0	F	N/A	MI	0	114	7.0	F	N/A	MI	0	183	1.0	F	N/A	MI	8	252	5.0	F	1700	SE	10
46	5.0	F	N/A	MI	0	115	8.0	F	N/A	MI	0	184	3.0	F	N/A	MI	26	253	6.0	F	1700	SE	55
47	3.0	F	N/A	MI	0	116	5.0	F	N/A	MI	0	185	2.0	F	N/A	MI	0	254	4.0	F	1700	SE	47
48	5.0	F	N/A	MI	0	117	5.0	F	N/A	MI	0	186	2.0	F	N/A	MI	5	255	5.0	F	1700	SE	68
49	8.0	F	N/A	MI	3	118	4.0	F	N/A	MI	0	187	4.0	F	N/A	MI	0	256	5.0	F	1700	SE	10
50	5.0	F	N/A	MI	0	119	8.0	F	N/A	MI	0	188	7.0	F	N/A	MI	39	257	4.0	F	1700	SE	5
51	2.5	M	N/A	MI	0	120	9.0	F	N/A	MI	0	189	8.0	F	N/A	MI	0	258	4.0	F	1700	SE	0
52	5.0	F	N/A	MI	0	121	9.0	F	N/A	MI	5	190	3.0	F	N/A	MI	3	259	2.0	F	1700	SE	47
53	5.0	F	N/A	MI	0	122	10.0	F	N/A	MI	0	191	2.0	M	N/A	MI	0	260	6.0	F	1700	SE	0
54	4.0	F	N/A	MI	3	123	3.0	F	N/A	MI	0	192	2.0	F	N/A	MI	0	261	3.0	M	1700	SE	10
55	8.0	F	N/A	MI	3	124	4.0	F	N/A	MI	0	193	2.0	M	N/A	MI	0	262	4.0	M	1700	SE	8
56	8.0	F	N/A	MI	3	125	6.0	F	N/A	MI	18	194	1.0	M	N/A	MI	0	263	3.0	F	1900	SE	0
57	4.0	F	N/A	MI	0	126	8.0	F	N/A	MI	3	195	1.0	F	N/A	MI	0	264	2.0	F	1900	SE	0
58	5.0	M	N/A	MI	0	127	2.0	F	N/A	MI	0	196	9.0	F	N/A	MI	0	265	2.0	F	1900	SE	0
59	3.0	M	N/A	MI	0	128	4.0	F	N/A	MI	0	197	0.5	M	N/A	MI	0	266	2.0	F	1900	SE	0
60	1.0	M	N/A	MI	0	129	1.0	F	N/A	MI	0	198	10.0	F	N/A	MI	0	267	2.0	F	1900	SE	0
61	2.5	F	N/A	MI	0	130	0.5	F	N/A	MI	0	199	9.0	F	N/A	MI	29	268	1.0	F	1900	SE	0
62	2.0	F	N/A	MI	0	131	3.0	F	N/A	MI	0	200	3.0	F	N/A	MI	0	269	7.5	F	1900	SE	3
63	4.0	F	N/A	MI	0	132	7.0	F	N/A	MI	0	201	2.0	F	N/A	MI	0	270	2.0	F	1900	SE	1
64	3.5	F	N/A	MI	0	133	8.0	F	N/A	MI	3	202	1.0	F	N/A	MI	0	271	3.0	F	1900	SE	0
65	1.0	F	N/A	MI	0	134	4.0	F	N/A	MI	5	203	4.0	F	N/A	MI	0	272	1.4	M	1900	SE	1
66	4.0	F	N/A	MI	0	135	2.0	F	N/A	MI	18	204	6.0	F	N/A	MI	0	273	5.0	F	1900	SE	0
67	6.0	F	N/A	MI	0	136	0.2	F	N/A	MI	4	205	8.0	F	N/A	MI	0	274	3.5	F	1900	SE	0
68	12.0	F	N/A	MI	0	137	6.0	F	N/A	MI	0	206	0.3	F	N/A	MI	0	275	2.0	M	1900	SE	0
69	2.0	F	N/A	MI	0	138	6.0	F	N/A	MI	3	207	0.3	F	N/A	MI	0						

No =animal number, Age =age in years; Alti =elevation (in metre) of the farm, N/A =not applicable; Sys = management system, MI =migratory, SE =Sedentary, EPG =eggs per gram of faeces; M =male, F = female.

Appendix Table 4.4.8
Longitudinal Survey of Farm/Flocks (1991-1992)
Monthly faecal *F. gigantica* egg counts of cattle in the hills

Description of animal			Farm location	EPG during different months												
No	Age	Sex	Altitude	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	11	M	400	13.0	2.6	2.6	1.3	1.3	0.0	5.2	1.3	6.5	9.1	2.6	0.0	2.6
2	9	F	400	3.9	0.0	0.0	0.0	0.0	0.0	3.9	1.3	5.2	2.6	0.0	0.0	0.0
3	8	M	450	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	5.2	9.1	0.0	0.0
4	5	M	450	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0
5	9	F	500	11.7	1.3	0.0	3.9	2.6	1.3	1.3	3.9	7.8	19.5	31.2	11.7	13.0
6	13	F	500	0.0	0.0	0.0	1.3	2.6	0.0	0.0	0.0	1.3	2.6	0.0	0.0	0.0
7	3	F	550	2.6	3.9	0.0	0.0	0.0	0.0	0.0	0.0	5.2	3.9	0.0	0.0	1.3
8	12	F	600	0.0	2.6	0.0	0.0	18.2	0.0	0.0	0.0	1.3	-	0.0	6.5	0.0
9	4	F	600	-	-	-	-	-	-	-	-	-	-	-	-	-
10	4	F	600	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	3	M	630	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9	F	650	0.0	0.0	0.0	0.0	2.6	0.0	1.3	1.3	-	2.6	0.0	0.0	0.0
13	4	M	700	0.0	2.6	37.7	0.0	-	0.0	1.3	3.9	3.9	-	-	-	-
14	3	M	700	0.0	1.3	0.0	0.0	1.3	2.6	3.9	0.0	1.3	3.9	7.8	0.0	7.8
15	7	F	700	-	-	-	-	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	15	M	700	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.7	19.4	1.3	0.0	0.0	0.0
17	3	F	700	0.0	2.6	0.0	0.0	2.6	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0
18	4	F	700	-	-	-	-	-	-	-	-	-	-	-	-	-
19	6	M	700	-	-	-	-	-	-	3.9	3.9	0.0	0.0	0.0	0.0	0.0
20	9	F	700	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	2.6	-	-
21	8	F	700	0.0	2.6	0.0	0.0	1.3	0.0	0.0	0.0	2.6	-	-	-	-
22	5	F	700	1.3	0.0	0.0	0.0	-	1.3	1.3	2.6	3.9	2.6	0.0	9.7	0.0
23	8	M	730	-	-	-	-	-	-	0.0	1.3	0.0	0.0	39.0	15.6	1.3
24	9	F	750	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0
25	4	M	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	0.0	0.0	19.5	0.0	0.0
26	5	F	1000	0.0	0.0	0.0	0.0	2.6	0.0	41.6	15.6	39.0	23.4	0.0	-	-
27	7	M	1000	2.6	1.3	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	1.3	0.0	0.0
28	9	F	1200	-	-	-	-	-	-	-	-	-	-	-	-	-
29	7	M	1250	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-
30	8	M	1250	-	-	-	-	-	-	-	-	-	-	-	-	-
31	5	M	1250	0.0	0.0	0.0	-	0.0	2.6	0.0	0.0	0.0	0.0	6.5	-	-
32	4	F	1300	0.0	0.0	0.0	0.0	0.0	0.0	2.6	2.6	0.0	0.0	0.0	0.0	0.0
33	7	M	1300	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3
34	9	M	1300	0.0	0.0	3.9	2.6	2.6	13.0	0.0	0.0	0.0	1.3	1.3	6.5	0.0
35	5	F	1300	-	-	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
36	5	M	1300	0.0	0.0	0.0	0.0	-	-	0.0	0.0	7.8	7.8	0.0	0.0	0.0
37	10	F	1300	-	-	-	-	-	-	-	-	-	-	-	-	-
38	3	M	1300	-	1.3	3.9	1.3	0.0	0.0	0.0	1.3	0.0	1.3	1.3	3.9	2.6
39	4	F	1300	0.0	0.0	0.0	0.0	2.6	5.2	0.0	0.0	-	0.0	11.7	2.6	0.0
40	12	F	1300	0.0	0.0	0.0	0.0	0.0	18.2	3.9	3.9	0.0	0.0	0.0	3.9	0.0
41	8	F	1300	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
42	6	F	1350	1.3	0.0	0.0	0.0	0.0	0.0	0.0	2.6	2.6	6.5	0.0	0.0	0.0
43	10	F	1350	0.0	1.3	0.0	1.3	1.3	2.6	0.0	0.0	1.3	3.9	7.8	0.0	7.8
44	7	M	1350	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	3.9	0.0
45	5	M	1350	-	-	-	-	0.0	0.0	0.0	0.0	0.0	-	-	-	-
46	6	F	1350	0.0	0.0	0.0	0.0	3.9	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
47	4	M	1400	1.3	1.3	3.9	1.3	6.5	0.0	0.0	0.0	5.2	2.6	0.0	-	-
48	9	M	1400	6.5	0.0	0.0	0.0	13.0	0.0	0.0	0.0	0.0	0.0	-	6.5	-
49	10	M	1400	0.0	0.0	0.0	0.0	0.0	1.3	0.0	3.9	15.6	1.3	0.0	0.0	0.0
50	8	F	1400	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9
51	6	F	1450	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-
52	6	M	1500	0.0	0.0	1.3	13.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
53	8	M	1500	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-
54	5	F	1500	2.6	1.3	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	1.3	0.0	0.0
55	11	F	1500	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	1.3	-
56	8	F	1550	2.6	1.0	5.2	0.0	0.0	1.3	0.0	0.0	3.9	9.1	18.2	13.0	16.9
57	7	M	1600	-	-	-	-	-	-	-	-	-	-	-	-	-
58	8	F	1600	1.3	2.6	1.3	3.9	3.9	1.3	10.4	6.5	2.6	19.5	20.8	14.3	11.7
59	8	M	1750	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-
60	7	F	1750	1.3	0.0	10.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
OVERALL																
Number of animal examined				47	48	48	47	47	49	53	53	48	45	44	42	39
Number of animals positive				15	17	9	9	18	14	13	17	20	21	18	13	11
Percentage positive				31.9	35.4	18.8	19.1	38.3	28.6	24.5	32.1	41.7	46.7	40.9	31.0	28.2
Mean EPG				4.0	1.9	7.8	3.3	4.1	3.9	6.3	4.2	6.9	6.2	10.3	7.6	6.4
Standard error of mean				1.0	0.2	3.6	1.2	1.0	1.3	2.9	0.9	1.9	1.4	2.6	1.3	1.6
LOW ALTITUDE (below 1200 m)																
Number of animal examined				21	21	21	21	19	21	24	24	23	21	21	20	20
Number of animals positive				6	10	2	3	10	4	10	11	13	11	9	4	5
Percentage positive				28.6	47.6	9.5	14.3	52.6	19.0	41.7	45.8	56.5	52.4	42.9	20.0	25.0
Mean EPG				5.9	2.1	10.2	2.2	3.8	1.6	6.5	4.6	7.6	7.0	12.7	10.9	5.2
Standard error of mean				1.9	0.3	6.4	0.7	1.5	0.3	3.7	1.4	2.8	2.2	4.4	1.6	2.0
MID ALTITUDE (1200 m and above)																
Number of animal examined				26	27	27	26	28	28	29	29	25	24	23	22	19
Number of animals positive				9	7	7	6	8	10	3	6	7	10	9	9	6
Percentage positive				34.6	25.9	25.9	23.1	28.6	35.7	10.3	20.7	28.0	41.7	39.1	40.9	31.6
Mean EPG				2.7	1.5	4.3	3.9	4.6	4.8	5.6	3.5	5.6	5.5	7.9	6.2	7.4
Standard error of mean				0.7	0.2	1.1	1.7	1.2	1.8	2.0	0.7	1.7	1.7	2.4	1.4	2.2

- = Animals not available for sampling

Appendix Table 4.4.9
Longitudinal Survey of Farm/Flocks (1991-1992)
Monthly faecal *F. gigantica* egg counts of cattle in Tarahara and Itahari VDC area (Terai)

Description of animal			EPG during different months												
No	Age	Sex	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	3.0	F	0.0	0.0	0.0	7.8	2.6	2.6	0.0	6.5	0.0	2.6	7.8	0.0	0.0
2	6.0	M	0.0	0.0	0.0	1.3	0.0	0.0	0.0	10.4	6.5	10.4	0.0	-	-
3	5.0	M	10.4	0.0	0.0	9.1	7.8	1.3	0.0	0.0	0.0	0.0	0.0	-	-
4	6.0	F	0.0	0.0	0.0	1.3	0.0	0.0	0.0	2.6	3.9	6.5	10.4	2.6	2.6
5	8.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	5.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	6.0	M	0.0	1.3	0.0	0.0	1.3	0.0	0.0	-	-	-	-	-	-
8	1.0	F	0.0	0.0	0.0	2.6	1.3	0.0	0.0	-	-	-	-	-	-
9	3.0	F	0.0	0.0	0.0	2.6	44.2	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	7.0	F	0.0	0.0	0.0	3.9	0.0	0.0	0.0	0.0	2.6	3.9	24.7	5.2	0.0
11	8.0	F	0.0	0.0	0.0	3.9	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	7.0	M	0.0	0.0	1.3	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
13	8.0	M	0.0	0.0	2.6	5.2	0.0	0.0	0.0	0.0	-	-	-	-	-
14	0.5	M	0.0	0.0	0.0	3.9	2.6	0.0	0.0	0.0	-	-	-	-	-
15	0.5	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-
16	3.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	3.0	F	0.0	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	3.0	M	0.0	0.0	5.2	0.0	24.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	3.0	F	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20	14.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	3.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	10.4	18.2	10.4	18.2
22	3.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	4.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	5.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	1.3	11.7	0.0
25	1.0	M	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	-	-	-	-	-
26	10.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-
27	1.5	F	0.0	0.0	-	0.0	0.0	0.0	0.0	-	-	-	-	-	-
28	10.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-
29	4.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-
30	6.0	M	0.0	0.0	0.0	0.0	3.9	0.0	0.0	1.3	2.6	11.7	19.5	2.6	0.0
31	1.5	F	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	-	-
32	2.0	F	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0
33	8.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
34	1.5	F	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
35	6.0	M	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
36	12.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
37	3.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
38	3.0	M	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
39	0.6	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40	7.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
41	12.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
42	2.0	F	0.0	0.0	0.0	0.0	0.0	0.0	6.5	0.0	0.0	0.0	0.0	0.0	2.6
43	1.5	F	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-
44	4.0	M	0.0	0.0	0.0	0.0	0.0	0.0	1.3	23.4	18.6	16.9	0.0	0.0	0.0
45	4.0	M	0.0	0.0	0.0	18.2	0.0	0.0	14.3	0.0	0.0	0.0	0.0	0.0	0.0
46	15.0	F	0.0	0.0	3.9	36.4	16.9	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0
47	5.0	F	0.0	0.0	0.0	127.5	0.0	0.0	0.0	3.9	0.0	3.9	0.0	0.0	0.0
48	3.0	F	0.0	11.7	0.0	7.8	0.0	0.0	0.0	0.0	7.5	2.6	0.0	11.7	0.0
49	9.0	M	0.0	1.3	0.0	0.0	1.3	1.3	0.0	0.0	0.0	0.0	0.0	2.6	0.0
50	10.0	M	0.0	1.3	0.0	1.3	32.4	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0
51	1.5	M	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
52	1.5	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
Number of animal examined			52	52	51	52	52	51	51	47	41	41	41	37	36
Number of animals positive			2	4	5	18	14	4	7	6	7	10	6	7	3
Percentage positive			3.8	7.7	9.8	34.6	26.9	7.8	13.7	12.8	17.1	24.4	14.6	18.9	8.3
Mean EPG			5.9	3.9	2.9	13.4	10.4	1.6	4.5	8.0	6.5	7.0	13.7	6.7	7.8
Standard error of mean			3.2	2.3	0.7	6.8	3.6	0.3	1.7	3.1	2.0	1.5	3.2	1.5	4.2

- = Animals not available for sampling

Appendix Table 4.4.10
Longitudinal Survey of Farm/Flock (1991-1992)
Monthly faecal *F. gigantica* egg counts of cattle in Letang VDC area (Terai)

Description of animal			EPG during different months												
No	Age	Sex	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	7	F	6.5	0.0	0.0	2.6	1.3	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0
2	6	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	3.9	2.6	0.0	0.0	0.0
3	6	M	0.0	0.0	0.0	6.5	0.0	1.3	6.5	46.8	11.7	2.6	0.0	0.0	0.0
4	8	M	0.0	0.0	0.0	5.2	2.6	0.0	2.6	0.0	0.0	3.9	0.0	9.1	0.0
5	8	M	1.3	0.0	0.0	1.2	9.1	0.0	0.0	0.0	0.0	0.0	-	-	-
6	5	M	3.9	0.0	0.0	0.0	2.6	0.0	1.3	0.0	0.0	1.3	-	-	-
7	5	M	0.0	0.0	0.0	3.9	5.2	0.0	2.6	1.3	0.0	2.6	0.0	1.3	0.0
8	2	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.2	6.5	1.3	0.0
9	2	F	0.0	0.0	0.0	1.3	1.3	11.7	3.9	1.3	3.9	0.0	0.0	0.0	0.0
10	1.5	F	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	1.3	2.6	0.0	2.6
11	6	M	0.0	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0
12	6	M	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0
13	10	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6
14	10	M	1.3	0.0	0.0	1.3	1.3	1.3	0.0	0.0	2.6	1.3	0.0	0.0	3.9
15	6	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0
16	11	M	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	11	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	1.5	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-
19	1	M	0.0	0.0	0.0	0.0	0.0	0.0	3.9	-	2.6	1.3	-	-	6.5
20	11	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	1.3	0.0
21	7	M	1.3	0.0	0.0	2.6	2.6	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0
22	7	F	1.3	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	1.5	M	0.0	0.0	1.3	0.0	1.3	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0
24	8	M	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	1.3	1.3	0.0	0.0
25	8	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.6	0.0	0.0
26	6	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0
27	6	F	3.9	0.0	-	3.9	2.6	26.0	0.0	0.0	0.0	0.0	2.6	0.0	1.2
28	1.5	F	0.0	0.0	0.0	0.0	2.6	0.0	1.3	0.0	0.0	0.0	2.6	3.9	0.0
29	7	F	0.0	0.0	0.0	10.4	7.8	1.3	0.0	0.0	0.0	0.0	0.0	0.0	-
30	1	M	0.0	0.0	0.0	0.0	15.6	0.0	0.0	0.0	0.0	0.0	6.5	6.5	-
31	9	F	0.0	0.0	0.0	2.6	2.6	0.0	0.0	0.0	0.0	0.0	2.6	7.8	3.9
32	6	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	2.6	0.0
33	7	F	0.0	2.6	0.0	0.0	16.9	1.3	1.3	0.0	0.0	0.0	0.0	0.0	0.0
34	0.4	F	0.0	-	1.3	6.5	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
35	11	M	0.0	0.0	0.0	5.2	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0
36	10	M	0.0	0.0	0.0	0.0	1.3	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
37	12	F	0.0	-	-	7.8	3.9	0.0	-	-	-	-	-	-	-
38	7	M	0.0	0.0	0.0	0.0	1.3	3.9	0.0	0.0	0.0	0.0	0.0	1.3	-
39	7	M	0.0	0.0	0.0	0.0	7.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
40	1	M	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-
41	8	M	0.0	1.3	0.0	0.0	2.6	2.6	1.3	0.0	0.0	0.0	6.5	3.9	-
42	8	M	1.3	1.3	0.0	2.6	1.3	5.2	1.3	0.0	0.0	0.0	0.0	0.0	0.0
43	6	F	0.0	0.0	0.0	0.0	1.3	1.3	0.0	0.0	0.0	0.0	0.0	0.0	1.3
44	4	M	0.0	0.0	0.0	3.9	2.6	3.9	0.0	0.0	0.0	-	-	-	-
45	0.4	M	0.0	2.6	0.0	1.3	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
46	6.0	F	0.0	-	-	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	2.6	0.0
Number of animal examined			46	43	43	46	46	46	45	44	45	43	39	39	34
Number of animals positive			9	4	3	22	23	15	11	4	5	13	14	12	7
Percentage positive			19.6	9.3	7.0	47.8	50.0	32.6	24.4	9.1	11.1	30.2	35.9	30.8	20.6
Mean EPG			2.6	2.0	1.7	3.7	4.2	4.4	2.5	12.7	4.9	2.1	4.3	3.6	3.1
Standard error of mean			1.1	0.8	0.4	0.5	0.7	1.3	1.5	5.6	1.5	0.7	1.0	1.1	0.9

- = Animals not available for sampling

Appendix Table 4.4.11
Longitudinal Survey of Farm/Flock (1991-1992)
Monthly faecal *F. gigantica* egg counts of cattle in Jhumka and Bhantabari VDCs area (Terai)

Description of animal			EPG during different months												
No	Age	Sex	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	12.0	F	54.6	0.0	0.0	1.3	2.6	0.0	0.0	9.1	0.0	0.0	0.0	2.6	3.9
2	5.0	M	45.5	0.0	0.0	20.8	2.6	0.0	0.0	1.3	0.0	0.0	20.8	48.1	1.3
3	5.0	M	15.6	0.0	0.0	1.3	1.3	0.0	0.0	2.6	0.0	0.0	13.0	65.0	13.0
4	7.0	F	35.1	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	0.0
5	14.0	F	49.4	1.3	1.3	0.0	0.0	0.0	9.1	1.3	9.1	12.5	3.9	1.3	0.0
6	8.0	M	2.6	0.0	0.0	37.4	19.5	0.0	0.0	0.0	61.1	0.0	0.0	2.6	0.0
7	6.0	M	14.3	0.0	0.0	7.8	9.1	3.9	0.0	-	1.3	2.6	2.6	5.2	0.0
8	4.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	1.0	F	0.0	0.0	0.0	9.1	7.8	5.2	0.0	0.0	0.0	0.0	3.9	1.3	0.0
10	8.0	F	0.0	0.0	0.0	0.0	0.0	6.5	0.0	0.0	19.4	13.0	26.0	0.0	0.0
11	7.0	F	0.0	0.0	0.0	0.0	0.0	6.5	0.0	0.0	0.0	0.0	2.6	0.0	0.0
12	7.0	M	0.0	1.3	3.9	2.6	0.0	0.0	0.0	0.0	0.0	3.9	2.6	3.9	0.0
13	6.0	M	0.0	0.0	0.0	0.0	1.3	13.0	0.0	2.6	0.0	0.0	1.3	0.0	0.0
14	12.0	F	6.5	0.0	-	-	-	2.6	0.0	-	5.2	0.0	0.0	0.0	-
15	7.0	M	0.0	0.0	0.0	1.3	1.3	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	6.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	9.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	0.0	11.7
18	10.0	M	0.0	1.3	3.9	0.0	0.0	0.0	0.0	0.0	0.0	1.4	3.9	0.0	0.0
19	9.0	M	0.0	0.0	-	0.0	0.0	0.0	20.8	-	0.0	0.0	0.0	0.0	-
20	3.0	F	0.0	0.0	-	0.0	0.0	1.3	0.0	-	0.0	6.5	10.4	0.0	-
21	1.0	M	0.0	0.0	0.0	0.0	0.0	0.0	1.3	-	0.0	0.0	-	-	-
22	10.0	F	0.0	0.0	-	0.0	0.0	0.0	0.0	-	6.5	0.0	-	-	-
23	5.0	M	0.0	2.6	13.0	19.5	15.6	-	0.0	-	2.6	2.6	1.3	1.3	3.9
24	0.4	M	0.0	1.3	0.0	0.0	0.0	-	0.0	-	0.0	0.0	0.0	-	0.0
25	4.0	M	0.0	1.3	2.6	70.2	0.0	-	0.0	-	-	-	1.3	10.4	0.0
26	6.0	F	0.0	3.9	15.6	75.4	0.0	-	-	-	-	-	0.0	-	-
27	5.0	F	0.0	0.0	0.0	0.0	0.0	-	0.0	-	0.0	3.9	1.3	19.5	13.0
28	1.0	F	2.6	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	-	2.6	13.0	-
29	12.0	F	0.0	0.0	0.0	68.9	3.9	-	-	0.0	0.0	-	0.0	0.0	0.0
30	7.0	M	0.0	1.3	7.8	15.6	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31	1.0	F	1.3	0.0	1.3	55.5	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32	7.0	F	7.8	2.6	13.0	72.8	0.0	3.9	0.0	0.0	-	0.0	0.0	0.0	0.0
33	6.0	F	0.0	0.0	0.0	2.6	1.3	0.0	0.0	0.0	-	3.9	3.9	13	-
34	2.5	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	2.6	6.5	6.5
35	7.0	M	0.0	0.0	0.0	59.8	2.6	0.0	-	-	-	-	-	-	-
36	8.0	F	0.0	0.0	1.3	62.4	15.6	-	-	-	0.0	9.1	9.1	1.3	3.9
37	10.0	F	0.0	0.0	0.0	76.7	2.6	-	-	-	0.0	0.0	0.0	0.0	0.0
38	6.0	M	0.0	0.0	-	1.3	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	9.1
39	8.0	M	0.0	1.3	3.9	9.1	0.0	0.0	1.3	0.0	0.0	2.6	3.9	0.0	0.0
40	9.0	M	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	2.6	9.1	6.5
41	12.0	M	0.0	0.0	0.0	6.5	10.4	0.0	0.0	-	10.4	6.5	20.8	0.0	0.0
42	10.0	M	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	2.6	1.3	0.0
43	7.0	F	0.0	0.0	-	0.0	0.0	0.0	-	-	-	-	-	-	-
44	9.0	M	0.0	0.0	0.0	0.0	1.3	1.3	-	0.0	0.0	0.0	0.0	0.0	0.0
45	6.0	M	0.0	0.0	0.0	9.1	2.6	0.0	-	2.6	0.0	2.6	1.3	0.0	0.0
46	10.0	F	1.3	0.0	0.0	2.6	2.6	7.8	-	0.0	0.0	0.0	0.0	0.0	5.2
47	3.0	F	0.0	0.0	0.0	2.6	0.0	2.6	-	1.3	0.0	0.0	0.0	0.0	0.0
48	6.0	F	0.0	0.0	0.0	-	0.0	1.3	0.0	0.0	0.0	2.6	1.3	0.0	0.0
49	3.0	M	1.3	0.0	0.0	1.3	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0
50	8.0	M	0.0	0.0	0.0	2.6	0.0	5.2	6.5	0.0	0.0	0.0	-	0.0	0.0
51	2.0	M	2.6	0.0	0.0	5.2	1.3	3.9	0.0	0.0	0.0	0.0	-	0.0	0.0
52	14.0	F	0.0	0.0	0.0	0.0	2.6	1.3	0.0	0.0	1.3	-	-	0.0	0.0
53	4.0	M	0.0	0.0	0.0	18.2	0.0	3.9	9.1	2.6	0.0	0.0	-	0.0	0.0
54	5.0	M	0.0	0.0	0.0	10.4	0.0	0.0	3.9	0.0	0.0	0.0	-	0.0	0.0
55	2.0	M	0.0	0.0	0.0	23.4	0.0	0.0	0.0	6.5	-	0.0	-	0.0	0.0
56	3.0	M	0.0	0.0	0.0	24.7	0.0	0.0	0.0	1.2	-	0.0	-	0.0	0.0
57	4.0	M	0.0	0.0	0.0	0.0	0.0	0.0	1.3	2.6	0.0	0.0	-	0.0	0.0
58	3.0	F	0.0	0.0	0.0	0.0	0.0	1.3	0.0	2.6	-	2.6	-	0.0	0.0
59	7.0	M	0.0	0.0	0.0	3.9	0.0	1.3	-	1.3	-	1.3	-	-	-
60	6.0	M	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	-	0.0	0.0
Number of animal examined			60	60	54	58	59	51	47	43	47	52	45	53	49
Number of animals positive			14	10	11	34	21	21	8	13	10	15	25	18	11
Percentage positive			23.3	16.7	20.4	58.6	35.6	41.2	17.0	30.2	21.3	28.8	55.6	34.0	22.4
Mean EPG			17.2	1.8	6.1	23.1	5.4	4.0	6.7	2.9	11.8	5.0	6.0	11.6	7.1
Standard error of mean			5.1	0.3	1.5	4.5	1.2	0.6	2.2	0.6	5.5	0.9	1.4	4.0	1.2

- = Animals not available for sampling

Appendix Table 4.4.12
Longitudinal Survey of Farm/Flock (1991-1992)
Monthly faecal *F. gigantica* egg counts of buffaloes in the hills

Description of animal			Farm location	EPG during different months												
No	Age	Sex	Altitude	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	14	F	350	-	-	-	-	-	-	-	-	-	-	-	-	-
2	3	F	450	15.6	7.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.0	19.5
3	5	F	450	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3
4	7	F	500	5.2	3.9	1.3	22.1	1.3	1.3	7.8	5.2	10.4	9.1	28.6	11.7	0.0
5	7	F	550	1.3	1.3	0.0	1.3	1.3	0.0	0.0	1.3	3.9	10.8	20.8	5.2	14.3
6	3	M	600	0.0	0.0	0.0	0.0	0.0	0.0	5.2	11.7	1.3	0.0	0.0	-	-
7	9	F	600	0.0	1.3	0.0	0.0	0.0	0.0	0.0	49.4	15.6	10.4	0.0	2.6	0.0
8	11	F	600	6.5	2.6	3.9	0.0	0.0	-	0.0	15.6	6.5	10.4	9.1	10.4	11.7
9	3	F	600	0.0	1.3	1.3	2.6	3.9	0.0	10.8	2.6	0.0	5.2	53.3	65.0	17.2
10	4	F	600	6.5	1.3	0.0	0.0	0.0	0.0	1.3	5.2	0.0	0.0	0.0	1.3	11.7
11	8	F	600	0.0	0.0	1.3	1.3	2.6	0.0	0.0	0.0	0.0	0.0	0.0	3.9	7.8
12	6	F	600	1.3	0.0	0.0	0.0	0.0	0.0	5.2	23.4	1.3	-	-	-	-
13	3	F	650	0.0	0.0	0.0	0.0	0.0	-	15.6	3.9	1.3	1.3	1.3	6.5	13.0
14	9	F	650	1.3	6.5	1.3	1.3	5.2	0.0	0.0	16.9	3.9	10.8	15.6	0.0	1.3
15	11	F	650	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	11	F	650	0.0	0.0	0.0	0.0	0.0	2.6	2.6	1.3	0.0	0.0	0.0	6.5	0.0
17	7	F	650	7.8	0.0	5.2	0.0	0.0	0.0	0.0	0.0	0.0	9.1	24.7	6.5	2.6
18	4	F	700	0.0	6.5	0.0	0.0	2.6	3.9	0.0	10.4	15.6	0.0	1.3	11.7	0.0
19	3	F	700	75.4	0.0	40.0	10.4	5.2	1.3	-	1.3	1.3	0.0	5.2	3.9	0.0
20	8	F	700	-	-	-	-	-	-	-	-	-	-	-	-	-
21	3	F	700	-	-	-	-	-	-	-	-	-	-	-	-	-
22	8	F	700	1.3	0.0	0.0	0.0	1.3	1.3	0.0	0.0	37.7	0.0	2.6	0.0	17.2
23	8	F	700	3.9	1.3	0.0	0.0	0.0	0.0	3.9	13.0	10.4	9.1	1.3	-	7.8
24	11	F	700	10.4	0.0	2.3	5.2	3.9	2.6	0.0	1.3	1.3	0.0	5.2	0.0	-
25	7	F	750	27.3	1.3	0.0	3.9	6.5	0.0	0.0	5.2	7.5	1.3	5.2	3.9	7.8
26	3	F	750	2.6	0.0	3.9	10.4	0.0	0.0	0.0	6.5	1.3	0.0	0.0	1.3	0.0
27	13	F	1000	-	-	-	-	-	-	-	-	-	-	-	-	-
28	7	F	1000	3.9	1.3	0.0	1.3	2.6	0.0	0.0	1.3		5.2	0.0	5.2	0.0
29	9	F	1000	-	10.4	1.3	0.0	5.2	5.2	0.0	0.0	1.3	0.0	0.0	0.0	0.0
30	12	F	1000	1.3	0.0	0.0	0.0	0.0	1.3	0.0	63.7	3.9	1.3	-	-	-
31	9	F	1200	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32	4	F	1200	19.5	76.7	5.2	0.0	6.5	0.0	0.0	0.0	39.0	7.2	0.0	0.0	31.2
33	9	F	1200	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.9	2.6
34	8	F	1200	-	-	-	-	-	-	-	-	-	-	-	-	-
35	7	F	1200	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
36	6	F	1250	0.0	0.0	0.0	0.0	0.0	3.9	6.5	0.0	-	0.0	0.0	0.0	0.0
37	4	F	1250	0.0	1.3	0.0	0.0	0.0	1.3	-	-	-	-	-	-	-
38	12	F	1280	5.2	0.0	3.9	2.6	2.6	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0
39	4	F	1300	5.2	1.3	0.0	0.0	10.4	0.0	0.0	0.0	0.0	0.0	0.0	1.3	2.6
40	7	F	1300	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.8	10.4
41	3	F	1300	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	-	0.0	0.0	9.1	3.9
42	9	F	1300	-	-	-	-	-	-	-	-	-	-	-	-	-
43	6	F	1300	0.0	0.0	0.0	0.0	0.0	3.9	26.0	5.2	0.0	0.0	0.0	21.6	0.0
44	9	F	1300	1.3	0.0	0.0	0.0	0.0	9.1	-	-	0.0	-	-	-	-
45	6	F	1350	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0
46	10	F	1400	-	-	-	-	-	-	-	-	1.3	0.0	-	-	-
47	4	F	1450	16.9	1.3	1.3	1.3	5.2	11.7	0.0	10.4	6.5	9.1	11.7	-	-
48	3	F	1450	5.2	0.0	1.3	9.1	1.3	26.0	1.3	1.3	0.0	0.0	0.0	0.0	0.0
49	8	F	1450	0.0	-	0.0	-	0.0	3.9	0.0	0.0	10.4	20.8	0.0	0.0	13.0
50	10	F	1500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	2.6	10.4	6.5
51	4	F	1500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	-	1.3	3.9	9.1	11.7
52	9	F	1500	1.3	0.0	0.0	0.0	5.2	0.0	0.0	0.0	-	1.3	3.9	3.9	0.0
53	3	F	1500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.0	4.3	4.3	3.9	0.0	11.7
54	10	F	1600	0.0	0.0	0.0	1.3	3.9	0.0	1.3	0.0	-	0.0	7.2	0.0	2.6
55	12	F	1600	10.4	2.6	5.2	2.6	-	-	-	-	-	-	-	-	-
56	10	F	1600	-	-	-	-	-	-	-	-	-	-	-	-	-
57	12	F	1700	0.0	0.0	1.3	0.0	0.0	0.0	15.6	-	-	-	-	-	-
58	8	F	1700	5.2	3.9	9.1	1.3	11.7	13.0	3.9	0.0	0.0	6.5	6.5	15.6	0.0
59	12	F	1700	0.0	3.9	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-
60	10	F	1750	0.0	0.0	0.0	0.0	-	-	-	-	6.5	0.0	-	-	-
OVERALL																
Number of animal examined				51	51	52	51	50	48	47	46	42	47	43	41	41
Number of animals positive				25	20	17	17	21	17	14	24	23	20	20	25	23
Percentage positive				49.0	39.2	32.7	33.3	42.0	35.4	29.8	52.2	54.8	42.6	46.5	61.0	56.1
Mean EPG				9.7	6.9	5.8	5.2	4.3	6.7	9.8	11.4	6.3	5.8	11.5	9.6	9.1
Standard error of mean				3.0	3.6	2.4	1.7	0.6	2.0	2.9	2.8	2.2	1.4	3.0	2.5	1.4
LOW ALTITUDE (below 1200 m)																
Number of animal examined				25	26	26	26	26	24	25	26	25	25	24	22	22
Number of animals positive				16	13	10	10	12	9	8	19	17	12	13	16	13
Percentage positive				64.0	50.0	38.5	38.5	46.2	37.5	32.0	73.1	68.0	48.0	54.2	72.7	59.1
Mean EPG				10.7	3.6	6.7	8.6	3.5	2.6	11.7	12.5	4.3	4.8	14.1	9.9	10.2
Standard error of mean				4.5	0.8	3.9	3.3	0.5	0.5	2.8	3.4	1.1	1.6	4.3	3.7	1.6
MID ALTITUDE (1200 m and above)																
Number of animal examined				26	25	26	25	24	24	22	20	17	22	19	19	19
Number of animals positive				9	7	7	7	9	8	6	5	6	8	7	9	10
Percentage positive				34.6	28.0	26.9	28.0	37.5	33.3	27.3	25.0	35.3	36.4	36.8	47.4	52.6
Mean EPG				7.8	13.0	4.3	2.8	5.3	8.8	9.1	6.2	15.6	6.6	6.2	9.1	8.0
Standard error of mean				2.0	9.8	1.1	1.0	1.2	2.7	3.7	2.1	9.6	2.1	1.1	2.0	2.2

- = Animals not available for sampling

Appendix Table 4.4.13
Longitudinal Survey of Farm/Flock (1991-1992)
Monthly faecal *F. gigantica* egg counts of buffaloes in Tarahara and Itahari VDC area (Terai)

Description of animal			EPG during different months												
No	Age	Sex	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	9	F	2.6	6.5	16.9	1.3	5.2	5.2	3.9	0.0	3.9	0.0	13.0	6.5	24.7
2	11	F	9.1	1.3	27.3	5.2	11.7	1.3	-	-	-	-	-	-	-
3	1	M	3.9	6.5	7.8	2.6	2.6	3.9	-	-	-	-	-	-	-
4	11	F	2.6	3.9	5.2	2.6	1.3	5.2	40.3	13.0	0.0	0.0	3.9	13.0	28.6
5	1.5	F	1.3	0.0	1.3	0.0	0.0	7.8	7.8	40.3	0.0	9.2	0.0	1.3	3.9
6	10	F	1.3	1.3	1.3	5.2	18.2	1.3	1.3	0.0	6.5	1.3	2.6	3.9	65.0
7	6	F	0.0	0.0	0.0	2.6	5.2	5.2	6.5	0.0	0.0	0.0	6.5	0.0	0.0
8	2	F	0.0	0.0	0.0	9.1	3.9	0.0	15.6	1.3	0.0	0.0	13.0	6.5	13.0
9	7	F	0.0	0.0	0.0	6.5	10.4	7.8	7.8	11.7	5.2	6.5	0.0	2.6	6.5
10	2	F	6.5	10.4	1.3	2.6	1.3	3.9	-	-	-	-	-	-	-
11	1	M	2.6	1.3	2.6	10.4	0.0	5.2	1.3	22.1	0.0	0.0	1.3	-	-
12	7	F	0.0	0.0	0.0	0.0	102.7	10.4	0.0	100.1	18.2	13.0	20.8	-	2.6
13	8	F	0.0	0.0	0.0	0.0	3.9	2.6	0.0	0.0	-	-	-	-	-
14	13	F	2.6	2.6	6.5	6.5	33.8	0.0	0.0	0.0	14.3	37.7	1.3	9.1	6.5
15	8	F	0.0	0.0	0.0	0.0	26.0	2.6	0.0	0.0	1.3	0.0	3.5	0.0	0.0
16	8	F	1.3	1.3	1.3	0.0	0.0	0.0	0.0	0.0	2.6	0.0	2.6	15.6	34.1
17	10	F	2.6	3.9	10.4	0.0	0.0	0.0	13.0	7.8	2.6	0.0	0.0	0.0	0.0
18	10	F	0.0	0.0	0.0	1.3	1.3	3.9	6.5	2.6	0.0	0.0	5.2	2.6	20.8
19	10	F	0.0	0.0	0.0	2.6	15.6	0.0	1.3	0.0	0.0	0.0	3.9	0.0	2.6
20	14	F	6.5	6.5	10.4	6.5	3.9	3.9	7.8	6.5	3.9	1.3	2.6	0.0	2.6
21	9	M	3.9	1.3	0.0	0.0	39.0	1.3	3.9	18.2	2.6	-	11.7	0.0	0.0
22	6	F	0.0	0.0	3.9	0.0	1.3	0.0	20.8	9.1	0.0	9.2	2.6	3.9	6.5
23	12	F	0.0	0.0	2.6	0.0	1.3	0.0	1.3	0.0	0.0	16.9	0.0	0.0	9.1
24	9	F	0.0	0.0	11.7	0.0	5.2	0.0	0.0	2.6	0.0	0.0	44.2	22.1	11.7
25	6	M	0.0	0.0	1.3	7.8	83.2	19.5	16.9	23.4	0.0	2.6	0.0	0.0	3.9
26	6	M	0.0	0.0	2.6	11.7	57.2	0.0	0.0	79.3	2.6	2.6	22.1	0.0	0.0
27	7	F	0.0	0.0	1.3	0.0	0.0	0.0	0.0	22.1	0.0	0.0	6.5	0.0	0.0
28	4	F	0.0	0.0	3.9	0.0	0.0	0.0	-	-	-	-	-	-	-
29	8	F	0.0	0.0	0.0	0.0	2.6	22.1	0.0	23.4	0.0	0.0	2.6	2.6	1.3
30	9	F	0.0	0.0	1.3	0.0	16.9	11.7	11.7	39.0	0.0	7.8	13.0	7.8	0.0
31	5	F	0.0	0.0	0.0	1.3	9.2	2.6	1.3	26.0	19.5	2.6	0.0	0.0	0.0
32	0.6	F	5.2	3.7	0.0	0.0	5.2	18.2	13.0	16.9	6.5	9.2	0.0	0.0	0.0
33	0.8	M	23.4	11.7	0.0	0.0	0.0	2.6	23.4	5.2	0.0	2.6	0.0	0.0	0.0
34	0.8	F	2.6	1.3	0.0	0.0	1.3	0.0	31.2	0.0	1.3	0.0	0.0	0.0	2.6
35	10	F	0.0	0.0	2.6	2.6	5.2	3.9	11.7	22.1	65.0	0.0	1.3	6.5	0.0
36	9	F	0.0	0.0	0.0	0.0	0.0	6.5	14.3	22.1	0.0	0.0	5.2	11.7	0.0
37	7	F	0.0	0.0	0.0	1.3	3.9	7.8	11.7	9.1	0.0	18.2	7.8	19.5	2.6
38	0.6	M	0.0	0.0	0.0	0.0	1.3	0.0	-	-	-	-	-	-	-
39	8	F	0.0	0.0	0.0	0.0	2.6	87.1	2.6	0.0	3.9	0.0	0.0	2.6	0.0
40	0.6	F	0.0	0.0	2.6	1.3	26.0	1.3	5.2	0.0	2.6	0.0	0.0	2.6	0.0
41	9	F	1.3	1.3	1.3	0.0	1.3	1.3	0.0	-	-	-	-	-	-
42	9	F	1.3	2.6	3.9	0.0	0.0	2.6	1.3	-	0.0	11.7	0.0	3.9	0.0
43	10	F	6.5	3.9	0.0	3.9	2.6	-	-	-	-	-	-	-	-
Number of animal examined			43	43	43	43	43	42	37	35	35	34	35	33	34
Number of animals positive			19	18	24	21	34	29	27	23	17	16	23	19	19
Percentage positive			44.2	41.9	55.8	48.8	79.1	69.0	73.0	65.7	48.6	47.1	65.7	57.6	55.9
Mean EPG			4.6	4.0	5.5	4.5	15.1	8.9	10.5	22.8	9.6	9.5	8.6	7.6	13.1
Standard error of mean			1.1	0.7	1.2	0.7	4.0	2.9	1.8	4.9	3.6	2.2	2.0	1.4	3.6

- = Animals not available for sampling

Appendix Table 4.4.14
Longitudinal Survey of Farm/Flock (1991-1992)
Monthly faecal *F. gigantica* egg counts of buffaloes in Letang VDC area (Terai)

Description of animal			EPG during different months												
No	Age	Sex	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	9	F	1.3	0.0	0.0	19.5	13.0	0.0	0.0	0.0	0.0	0.0	3.9	5.2	3.9
2	2	M	0.0	0.0	0.0	2.6	2.6	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0
3	8	F	0.0	0.0	0.0	37.7	10.4	5.2	0.0	0.0	0.0	0.0	9.1	1.3	2.6
4	1.5	F	0.0	0.0	0.0	23.4	19.5	2.6	2.6	0.0	0.0	1.3	3.9	7.8	3.9
5	1	F	10.4	14.3	13.0	19.5	2.6	0.0	0.0	0.0	-	2.6	0.0	0.0	-
6	1	M	0.0	5.2	2.6	15.6	13.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0
7	11	F	19.5	16.9	7.8	24.7	11.7	11.7	3.9	7.8	3.9	2.6	2.6	2.6	3.9
8	7	F	0.0	0.0	0.0	20.8	7.8	6.5	0.0	0.0	0.0	0.0	2.6	5.2	14.3
9	2	F	0.0	0.0	2.6	39.0	19.5	42.9	2.6	3.9	2.6	3.9	3.9	6.5	3.9
10	1	F	0.0	0.0	0.0	11.7	6.5	15.6	10.4	3.9	10.4	5.2	5.2	10.9	6.5
11	6	F	0.0	2.6	1.3	6.5	5.2	5.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	8	F	0.0	6.5	6.5	57.2	16.9	1.3	6.5	26.0	13.0	0.0	0.0	0.0	0.0
13	0.5	M	35.1	156.0	35.1	48.1	16.9	13.0	6.5	13.0	66.3	26.0	13.0	0.0	0.0
14	11	F	1.3	10.4	61.1	11.7	42.9	11.7	0.0	0.0	0.0	-	3.9	3.9	13.0
15	0.5	M	0.0	6.5	19.5	68.9	150.8	26.0	-	6.5	-	-	3.9	1.3	0.0
16	14	F	0.0	0.0	11.7	35.1	41.6	14.3	2.6	1.3	0.0	9.1	24.6	1.3	5.2
17	0.7	F	0.0	0.0	0.0	1.3	0.0	0.0	-	0.0	-	-	-	-	-
18	6	F	0.0	0.0	0.0	18.2	26.0	0.0	0.0	0.0	0.0	2.6	41.2	6.5	13.0
19	0.8	F	2.6	10.4	0.0	2.6	0.0	0.0	-	-	-	-	-	-	-
20	8	F	0.0	6.5	0.0	23.4	23.4	6.5	0.0	0.0	0.0	2.6	9.1	3.9	0.0
21	0.8	M	0.0	0.0	0.0	13.0	3.9	2.6	-	-	0.0	0.0	0.0	0.0	0.0
22	8	M	0.0	0.0	2.6	16.9	10.4	3.9	-	-	-	-	-	5.2	0.0
23	0.8	M	0.0	0.0	0.0	3.9	13.0	22.1	-	-	-	-	-	0.0	0.0
24	11	F	0.0	-	-	24.7	0.0	28.6	13.0	5.2	3.9	3.9	11.7	0.0	0.0
25	0.6	M	1.3	-	-	1.3	5.2	0.0	0.0	0.0	0.0	0.0	-	-	-
26	2	F	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.2	11.7	13.0
27	14	F	3.9	5.2	123.5	5.2	16.9	0.0	0.0	-	0.0	0.0	3.9	2.6	2.6
28	7	F	0.0	0.0	20.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35.5	10.4	-
29	0.6	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
30	7	F	2.6	0.0	0.0	0.0	0.0	0.0	13.0	2.6	1.3	1.3	0.0	0.0	0.0
31	6	F	5.2	2.6	0.0	1.3	13.0	11.7	9.1	3.9	0.0	-	0.0	0.0	0.0
32	7	F	0.0	0.0	0.0	0.0	0.0	2.6	1.3	2.6	2.6	1.3	0.0	1.3	0.0
33	1.5	F	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-
34	12	F	0.0	0.0	0.0	0.0	2.6	2.6	2.6	1.3	7.8	0.0	0.0	0.0	0.0
35	0.4	M	0.0	0.0	31.2	0.0	0.0	-	-	-	-	-	-	-	-
36	10	F	1.3	1.3	1.3	33.8	9.1	-	0.0	7.8	3.9	1.3	0.0	6.5	3.9
37	6	F	1.3	3.9	101.4	49.4	13.0	0.0	-	-	-	-	-	-	-
38	0.5	M	0.0	1.3	3.9	3.9	10.4	15.0	-	-	-	-	-	-	-
39	9	F	6.5	15.6	7.8	27.3	0.0	0.0	0.0	6.5	13.0	0.0	3.9	2.6	6.5
40	6	F	0.0	1.3	0.0	2.6	16.9	13.0	-	-	-	-	-	-	-
41	12	F	3.9	5.2	0.0	5.2	2.6	0.0	1.3	1.3	5.2	5.2	1.3	5.2	10.4
42	9	F	0.0	2.6	2.6	0.0	0.0	0.0	0.0	0.0	0.0	7.8	1.3	0.0	0.0
43	0.6	F	6.5	14.3	0.0	0.0	1.3	1.3	2.6	-	-	-	-	-	-
44	7	F	9.1	0.0	0.0	10.4	13.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
45	12	F	48.1	6.5	0.0	6.5	7.8	2.6	0.0	0.0	0.0	15.6	0.0	0.0	0.0
Number of animal examined			45	43	43	45	45	43	34	34	32	32	34	35	33
Number of animals positive			18	21	19	35	33	25	14	15	12	16	20	21	15
Percentage positive			40.0	48.8	44.2	77.8	73.3	58.1	41.2	44.1	37.5	50.0	58.8	60.0	45.5
Mean EPG			9.0	14.1	24.0	19.8	17.3	10.4	5.6	6.2	11.2	5.8	9.5	4.9	7.1
Standard error of mean			3.0	7.0	7.8	2.9	4.4	2.0	1.1	1.6	4.9	1.6	2.5	0.7	1.1

- = Animals not available for sampling

Appendix Table 4.4.15
Longitudinal Survey of Farm/Flock (1991-1992)
Monthly faecal *F. gigantica* egg counts of buffaloes in Jhumka and Bhantabari VDC area (Terai)

Description of animal			EPG during different months												
No	Age	Sex	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	11.0	F	5.2	3.9	7.8	2.6	0.0	6.5	3.9	0.0	39.0	13.0	15.6	6.5	1.3
2	8.0	F	0.0	0.0	0.0	2.6	0.0	6.5	2.6	7.8	37.7	10.4	49.4	9.1	22.1
3	9.0	F	2.6	6.5	9.1	13.0	0.0	2.6	3.9	0.0	18.2	0.0	1.3	3.9	2.6
4	4.0	F	11.7	2.6	2.6	5.2	0.0	9.1	9.1	3.9	19.5	3.9	5.2	1.3	11.7
5	3.0	F	2.6	13.0	28.6	1.3	0.0	1.3	32.5	0.0	0.0	0.0	0.0	0.0	0.0
6	2.0	M	13.0	6.5	29.6	2.6	0.0	0.0	10.4	11.7	10.4	11.7	19.5	10.4	13.0
7	15.0	F	3.9	0.0	9.1	7.8	0.0	15.6	13.0	0.0	0.0	0.0	7.8	0.0	0.0
8	18.0	F	0.0	0.0	1.3	2.6	1.3	0.0	45.2	5.2	0.0	7.8	39.0	1.3	0.0
9	15.0	F	23.6	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	4.0	F	19.5	0.0	0.0	10.4	1.3	7.8	6.5	2.6	6.5	7.8	23.4	7.8	6.5
11	10.0	F	2.6	0.0	0.0	7.8	0.0	2.6	13.0	0.0	23.4	0.0	0.0	0.0	52.0
12	9.0	F	3.9	2.6	0.0	11.7	1.3	1.3	0.0	7.8	7.5	6.5	11.7	0.0	18.2
13	15.0	F	0.0	0.0	0.0	0.0	0.0	2.6	0.0	2.6	13.0	11.7	1.3	0.0	28.6
14	18.0	F	5.2	3.9	2.6	0.0	0.0	0.0	3.9	0.0	7.2	0.0	0.0	0.0	0.0
15	9.0	F	1.3	1.3	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	13.0	F	9.1	6.5	6.5	3.9	3.9	6.5	0.0	1.3	97.5	9.1	26.0	0.0	48.1
17	21.0	F	2.6	2.6	3.9	1.3	0.0	13.0	2.6	2.6	44.2	10.4	19.5	2.6	39.8
18	8.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	37.7	11.7	10.4	0.0	0.0
19	6.0	F	0.0	0.0	0.0	2.6	6.5	5.2	18.2	2.6	-	0.0	5.2	0.0	11.7
20	4.0	F	0.0	0.0	0.0	0.0	9.1	42.9	0.0	9.1	39.0	0.0	3.9	0.0	3.9
21	8.0	F	2.6	2.6	3.9	2.6	0.0	10.4	0.0	0.0	10.4	0.0	3.9	3.9	-
22	16.0	F	0.0	0.0	0.0	2.6	0.0	3.9	0.0	0.0	1.3	2.6	5.2	1.3	-
23	6.0	F	1.3	-	2.6	1.3	7.8	1.3	26.0	0.0	0.0	0.0	0.0	-	-
24	14.0	F	0.0	-	0.0	2.6	0.0	2.6	0.0	0.0	10.4	0.0	0.0	-	-
25	10.0	F	0.0	0.0	0.0	3.9	7.8	1.3	13.0	0.0	7.2	6.5	7.8	0.0	0.0
26	6.0	F	0.0	0.0	0.0	0.0	3.9	18.2	0.0	0.0	2.6	0.0	0.0	0.0	0.0
27	0.5	F	0.0	0.0	0.0	10.4	0.0	24.7	0.0	10.4	14.3	0.0	0.0	0.0	-
28	3.0	F	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	-	0.0	0.0	2.6	-
29	9.0	F	0.0	0.0	0.0	0.0	6.5	1.3	52.0	1.3	26.0	1.3	0.0	2.6	0.0
30	16.0	F	0.0	0.0	0.0	0.0	3.9	0.0	7.8	3.9	1.3	1.3	0.0	5.2	18.2
31	1.5	M	1.3	2.6	3.9	28.6	0.0	0.0	0.0	-	5.2	5.2	0.0	-	2.6
32	6.0	F	0.0	0.0	0.0	16.9	0.0	10.4	6.5	-	-	-	0.0	-	14.3
33	14.0	F	0.0	0.0	0.0	0.0	1.3	6.5	6.5	-	-	-	0.0	22.1	7.8
34	0.7	F	0.0	0.0	0.0	3.9	1.3	0.0	5.2	2.6	0.0	1.3	2.6	1.3	14.3
35	7.0	M	14.3	10.4	0.0	0.0	2.6	0.0	11.7	5.2	36.4	6.6	2.6	5.2	5.2
36	8.0	F	0.0	0.0	0.0	9.1	1.3	0.0	1.3	-	1.3	3.9	5.2	0.0	0.0
37	1.7	F	0.0	0.0	2.6	0.0	0.0	0.0	0.0	-	10.8	-	-	-	-
38	4.0	f	0.0	0.0	0.0	10.4	1.3	0.0	0.0	-	11.7	19.5	1.3	-	19.5
39	2.0	F	2.6	3.9	0.0	0.0	0.0	1.3	5.2	45.5	1.3	9.3	1.3	0.0	0.0
40	1.0	F	6.5	2.6	0.0	0.0	0.0	0.0	13.0	15.6	-	20.2	24.7	0.0	0.0
41	3.0	F	1.3	0.0	2.6	9.1	1.3	1.3	0.0	0.0	-	1.3	3.9	0.0	0.0
42	14.0	F	0.0	0.0	0.0	0.0	0.0	2.6	24.7	0.0	0.0	-	-	-	-
43	0.6	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-
44	8.0	F	0.0	0.0	0.0	53.3	0.0	0.0	0.0	-	1.3	1.3	3.9	0.0	0.0
45	14.0	F	0.0	0.0	0.0	0.0	0.0	1.3	0.0	-	-	-	-	-	-
46	4.0	F	2.6	2.6	13.0	15.6	3.9	0.0	2.6	6.5	3.9	1.3	7.8	0.0	-
47	9.0	F	0.0	0.0	-	0.0	6.5	0.0	6.5	11.7	-	-	3.9	0.0	0.0
48	8.0	F	0.0	0.0	3.9	9.1	0.0	0.0	0.0	-	-	0.0	3.9	0.0	0.0
49	15.0	F	0.0	0.0	7.8	18.2	0.0	10.4	10.4	27.3	84.5	6.5	3.9	3.9	5.2
50	2.0	F	0.0	0.0	3.9	0.0	0.0	9.1	0.0	0.0	1.3	7.2	0.0	0.0	-
51	1.0	F	0.0	0.0	20.8	0.0	0.0	0.0	0.0	16.9	-	-	-	-	-
52	8.0	F	0.0	0.0	0.0	-	3.9	0.0	0.0	0.0	0.0	2.6	0.0	1.3	0.0
Number of animal examined			52	50	51	51	52	52	52	42	41	44	47	42	39
Number of animals positive			22	16	21	31	30	31	28	23	33	28	30	18	21
Percentage positive			42.3	32.0	41.2	60.8	57.7	59.6	53.8	54.8	80.5	63.6	63.8	42.9	53.8
Mean EPG			6.3	4.6	8.0	8.8	3.8	7.5	13.6	9.0	19.4	7.2	10.7	5.1	16.5
Standard error of mean			1.3	0.8	1.9	1.9	0.5	1.6	2.7	2.0	3.5	1.0	2.2	1.2	3.1

- = Animals not available for sampling

Appendix Table 4.4.16
Longitudinal Survey of Farm/Flock (1991-1992)
Monthly faecal *F. gigantica* egg counts of goats in the hills

Description of animal			Farm location	EPG during different months												
No	Age	Sex	Altitude	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	4.0	F	350	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	3.9	2.6	2.6
2	5.0	F	350	0.0	0.0	0.0	1.3	2.6	0.0	0.0	1.3	0.0	0.0	-	-	-
3	3.0	F	400	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	2.6	1.3	1.3
4	1.0	M	450	-	0.0	0.0	0.0	0.0	1.3	2.6	0.0	0.0	0.0	0.0	0.0	0.0
5	5.0	F	450	1.3	2.6	2.6	6.5	5.2	0.0	0.0	0.0	0.0	-	-	2.6	0.0
6	4.0	F	450	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	0.0	0.0
7	5.0	F	450	0.0	0.0	0.0	0.0	0.0	1.3	2.6	2.6	2.6	3.9	3.9	7.8	1.3
8	5.0	F	600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9
9	1.0	F	600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0
10	4.0	F	600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	2.0	F	600	-	-	-	-	-	-	-	-	-	-	-	-	-
12	0.6	M	600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-
13	2.0	M	630	0.0	0.0	0.0	0.0	0.0	0.0	C.0	0.0	0.0	0.0	0.0	0.0	0.0
14	2.0	F	630	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	-	0.0
15	2.5	M	630	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
16	1.0	M	650	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	-
17	1.0	M	650	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0
18	5.0	F	650	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-
19	2.0	M	700	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
20	3.0	F	700	3.9	1.3	2.6	3.9	3.9	0.0	0.0	0.0	0.0	2.6	0.0	-	-
21	3.0	F	700	0.0	1.3	6.5	-	0.0	-	-	-	-	-	-	0.0	0.0
22	3.0	F	700	10.4	3.9	0.0	0.0	0.0	0.0	0.0	0.0	1.3	2.6	2.6	2.6	-
23	1.0	M	700	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	4.0	F	1000	-	0.0	0.0	0.0	1.3	0.0	0.0	0.0	7.8	0.0	0.0	0.0	0.0
25	3.0	F	1000	-	-	-	-	-	-	-	-	-	-	-	-	-
26	7.0	F	1000	-	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	-	0.0	0.0	0.0
27	3.0	F	1000	-	0.0	0.0	0.0	2.6	2.6	3.9	1.3	0.0	0.0	0.0	0.0	0.0
28	7.0	F	1100	-	-	-	-	-	-	-	-	-	-	-	-	-
29	4.0	F	1200	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	2.6	0.0	0.0	0.0
30	5.0	F	1250	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9.0	F	1250	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	2.6	1.3
32	4.0	F	1250	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33	3.0	F	1250	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
34	4.0	F	1250	-	-	-	-	-	-	-	-	-	-	-	-	-
35	3.0	F	1300	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.5	5.2	3.9
36	4.0	F	1300	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
37	6.0	F	1300	0.0	1.3	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
38	8.0	F	1300	1.3	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	-	-	-	-
39	3.0	F	1300	-	0.0	0.0	3.9	3.9	0.0	3.9	0.0	0.0	0.0	0.0	-	-
40	3.0	F	1300	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	-	0.0	0.0	0.0
41	4.0	F	1300	1.3	1.3	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	2.6	2.6	0.0
42	1.0	M	1300	2.6	2.6	0.0	-	0.0	0.0	0.0	0.0	0.0	-	-	-	-
43	1.0	F	1350	0.0	0.0	5.2	0.0	-	-	-	-	-	-	-	-	-
44	7.0	F	1400	1.3	3.9	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0
45	5.0	F	1400	0.0	0.0	0.0	0.0	1.3	1.3	6.5	5.2	-	2.6	0.0	0.0	0.0
46	3.0	F	1400	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
47	4.0	F	1400	0.0	0.0	0.0	3.9	5.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	1.5	F	1400	-	0.0	0.0	0.0	1.3	1.3	1.3	0.0	0.0	1.3	3.9	10.8	2.6
49	4.0	F	1400	0.0	0.0	0.0	0.0	0.0	2.6	2.6	0.0	2.6	1.3	2.6	2.6	2.6
50	5.0	F	1450	0.0	0.0	0.0	0.0	0.0	3.9	2.5	0.0	2.6	6.5	2.6	0.0	1.3
51	4.0	F	1450	0.0	0.0	0.0	1.3	6.5	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0
52	5.0	F	1500	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	3.9	6.5	3.9	0.0
53	8.0	F	1500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
54	4.0	F	1500	1.3	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
55	4.0	F	1550	-	-	-	-	-	-	-	-	-	-	-	-	-
56	4.0	F	1550	0.0	0.0	1.3	0.0	5.2	0.0	0.0	0.0	0.0	-	-	-	-
57	7.0	F	1550	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	1.3
58	1.0	M	1700	0.0	3.9	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-
59	4.0	F	1700	0.0	0.0	1.3	0.0	0.0	0.0	0.0	5.2	3.9	0.0	-	0.0	0.0
60	3.0	F	1700	1.3	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	-	-
OVERALL																
Number of animal examined				48	54	54	51	53	51	52	50	47	42	39	38	38
Number of animals positive				11	9	9	8	12	9	8	7	7	10	10	11	10
Percentage positive				22.9	16.7	16.7	15.7	22.6	17.6	15.4	14.0	14.9	23.8	25.6	28.9	26.3
Mean EPG				2.6	2.5	3.0	3.3	3.5	1.9	3.0	2.8	3.2	2.7	3.8	4.1	2.2
Standard error of mean				0.8	0.4	0.6	0.6	0.5	0.3	0.5	0.6	0.8	0.5	0.5	0.8	0.3
LOW ALTITUDE (below 1200 m)																
Number of animal examined				21	25	25	23	25	24	24	25	23	20	17	18	17
Number of animals positive				4	4	3	3	6	4	3	4	3	4	4	5	4
Percentage positive				19.0	16.0	12.0	13.0	24.0	16.7	12.5	16.0	13.0	20.0	23.5	27.8	23.5
Mean EPG				4.2	2.3	3.9	3.9	3.1	1.6	3.0	1.6	3.9	2.3	3.3	3.4	2.3
Standard error of mean				1.9	0.5	1.1	1.2	0.5	0.3	0.4	0.3	1.6	0.5	0.3	1.0	0.5
MID ALTITUDE (1200 m and above)																
Number of animal examined				27	29	29	28	28	27	28	25	24	22	22	20	21
Number of animals positive				7	5	6	5	6	5	5	3	4	6	6	6	6
Percentage positive				25.9	17.2	20.7	17.9	21.4	18.5	17.9	12.0	16.7	27.3	27.3	30.0	28.6
Mean EPG				1.7	2.6	2.6	2.9	3.7	2.1	3.0	4.3	2.6	3.0	4.1	4.6	2.2
Standard error of mean				0.2	0.5	0.6	0.5	0.8	0.5	0.8	0.7	0.5	0.7	0.7	1.2	0.4

- = Animals not available for sampling

Appendix Table 4.4.17
Longitudinal Survey of Farm/Flock (1991-1992)
Monthly faecal *F. gigantica* egg counts of goats in Letang VDC (Terai)

Description of animal			EPG during different months												
No	Age	Sex	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	4.0	F	0.0	1.3	1.3	1.3	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	1.5	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	5.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
4	1.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
5	1.0	F	0.0	0.0	6.5	0.0	0.0	0.0	0.0	-	-	-	-	-	-
6	1.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	2.6
7	1.5	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	1.0	F	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	1.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	2.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	1.0	M	6.5	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	1.5	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	1.0	M	0.0	0.0	0.0	3.9	2.6	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	4.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	1.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.5	M	0.0	-	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	1.5	F	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-
18	4.0	F	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	9.1	5.2
19	2.0	M	1.3	13.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.0	40.3	
20	1.0	F	0.0	15.6	0.0	0.0	0.0	1.3	0.0	2.6	2.6	3.9	2.6	29.9	0.0
21	9.0	F	2.6	3.9	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.4	6.5
22	1.0	F	0.0	0.0	3.9	0.0	0.0	-	-	-	-	-	-	-	-
23	0.3	M	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
24	0.3	F	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	8.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	2.6
26	0.5	F	2.6	1.3	3.9	1.3	3.9	2.6	3.9	1.6	2.6	0.0	0.0	1.3	0.0
27	1.0	M	3.9	2.6	0.0	0.0	0.0	0.0	1.3	0.0	1.3	2.6	1.3	6.5	2.6
28	1.5	F	2.6	2.6	1.3	1.3	13.0	0.0	-	-	-	-	-	-	-
29	3.0	F	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30	3.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31	3.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32	5.0	F	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-
33	1.0	M	0.0	0.0	5.2	2.6	13.0	26.0	-	-	-	-	-	-	-
34	1.5	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
35	2.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
36	4.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
37	5.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
38	2.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
39	2.5	F	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40	1.0	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
41	5.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
42	6.0	F	6.5	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
43	5.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
44	4.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
45	5.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
46	3.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0
47	5.0	F	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
48	7.0	F	3.9	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
49	8.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50	6.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
51	3.0	F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
52	1.0	M	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	-	-
53	5.0	F	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0
54	6.0	F	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0
55	1.4	M	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0
Number of animal examined			55	54	54	53	55	51	45	48	48	48	47	42	39
Number of animals positive			8	8	10	6	6	6	2	2	3	2	4	8	5
Percentage positive			14.5	14.8	18.5	11.3	10.9	11.8	4.4	4.2	6.3	4.2	8.5	19.0	12.8
Mean EPG			3.7	4.4	3.0	2.4	5.9	6.1	2.6	2.1	2.2	3.3	5.2	13.2	3.9
Standard error of mean			0.6	1.3	0.6	0.5	2.1	3.7	0.9	0.4	0.4	0.5	2.3	4.7	0.7

- = Animals not available for sampling

Appendix Table 4.4.18
Longitudinal Survey of Farm/Flock (1991-1992)
Monthly faecal *F. gigantica* egg counts of tracer calves (cattle) in the hills

Description of animal			Farm location	EPG during different months												
No	Age	Sex	Altitude	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	1.0	M	400	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.8	2.6
2	1.0	F	400	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	3.9	0.0
3	1.0	F	450	0.0	0.0	0.0	0.0	3.9	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.0	F	450	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	2.0	M	450	5.2	0.0	-	-	-	-	-	-	-	-	-	-	-
6	1.0	M	450	-	-	-	-	-	-	-	-	-	-	-	-	-
7	1.0	F	450	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0
8	1.0	F	450	0.0	1.3	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	1.0	F	500	0.0	2.6	0.0	1.3	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0
10	1.0	F	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.5	0.0	0.0	0.0	1.3
11	1.0	M	500	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0
12	1.5	F	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.8	2.6	0.0
13	2.0	M	600	0.0	2.6	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	1.0	M	600	0.0	2.6	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	2.0	F	630	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	2.0	F	630	0.0	0.0	0.0	1.3	1.3	0.0	0.0	0.0	5.2	0.0	0.0	0.0	0.0
17	1.0	F	650	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	1.3
18	2.0	M	650	0.0	0.0	0.0	0.0	1.3	2.6	0.0	0.0	0.0	2.6	0.0	1.3	1.3
19	1.5	F	650	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	3.9	0.0	0.0	0.0	0.0
20	1.0	M	700	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6
21	1.0	M	700	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	-
22	2.0	F	700	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	2.0	M	700	0.0	1.3	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0
24	2.0	F	700	0.0	2.6	0.0	0.0	5.2	0.0	0.0	0.0	0.0	0.0	0.0	-	-
25	1.0	F	700	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0
26	1.5	M	750	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	1.3	0.0	0.0	0.0
27	1.5	F	750	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28	2.0	M	750	-	-	-	-	-	-	-	-	-	-	-	-	-
29	1.0	F	750	0.0	0.0	0.0	0.0	0.0	2.6	5.2	0.0	0.0	0.0	0.0	3.9	0.0
30	2.0	F	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-
31	2.0	F	1000	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	3.2	0.0	0.0	0.0
32	2.0	F	1150	3.9	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33	2.0	F	1200	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	-	0.0	0.0	0.0	1.3
34	2.0	F	1200	0.0	1.3	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	3.9	1.3
35	1.5	F	1200	0.0	0.0	0.0	-	2.6	-	-	-	-	-	-	-	-
36	2.0	F	1300	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	3.9	0.0	2.6	0.0
37	2.0	F	1300	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	3.9	0.0	0.0	0.0
38	2.0	F	1300	0.0	6.5	0.0	0.0	0.0	-	3.9	0.0	0.0	0.0	0.0	5.2	1.3
39	2.0	F	1300	0.0	5.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40	1.5	M	1300	0.0	0.0	0.0	0.0	2.6	0.0	1.3	0.0	0.0	0.0	0.0	0.0	-
41	1.0	F	1300	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	3.9	5.2	0.0
42	2.0	F	1300	0.0	1.3	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
43	2.0	F	1300	0.0	0.0	0.0	-	0.0	0.0	-	-	-	-	-	-	0.0
44	1.5	F	1350	0.0	0.0	0.0	2.6	6.5	0.0	0.0	0.0	0.0	0.0	-	-	-
45	2.0	F	1350	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6
46	1.5	F	1350	0.0	0.0	6.5	1.3	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
47	2.0	M	1350	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	1.3	0.0
48	2.0	M	1350	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	-	-	-	-
49	1.0	F	1350	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0
50	1.0	F	1350	0.0	0.0	0.0	0.0	6.5	0.0	0.0	0.0	0.0	0.0	0.0	1.3	-
51	1.0	M	1350	-	-	0.0	0.0	0.0	0.0	0.0	0.0	-	-	0.0	1.3	0.0
52	1.5	M	1400	2.6	0.0	0.0	0.0	1.3	0.0	-	-	-	0.0	0.0	0.0	0.0
53	2.0	F	1400	9.1	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
54	1.0	F	1450	0.0	0.0	1.3	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
55	1.0	M	1500	6.5	0.0	0.0	0.0	-	-	-	-	-	-	-	-	-
56	2.0	M	1500	6.5	0.0	0.0	0.0	-	0.0	-	-	-	-	-	-	-
57	1.0	F	1500	0.0	0.0	0.0	0.0	2.6	0.0	-	-	-	-	-	-	-
58	2.0	F	1700	0.0	0.0	1.3	0.0	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0	-
59	1.0	M	1750	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	-	-
OVERALL																
Number of animal at risk				56	49	42	37	37	26	22	23	34	40	38	30	26
Number of animals positive				7	11	3	3	13	7	3	0	4	6	12	7	4
Percentage positive				12.5	22.4	7.1	8.1	35.1	26.9	13.6	0.0	11.8	15.0	31.6	23.3	15.4
LOW ALTITUDE (below 1200 m)																
Number of animal at risk				30	27	23	23	21	17	12	14	22	22	20	15	16
Number of animals positive				3	7	0	2	6	4	1	0	3	3	4	3	2
Percentage positive				10.0	25.9	0.0	8.7	28.6	23.5	8.3	0.0	13.6	13.6	20.0	20.0	12.5
MID ALTITUDE (1200 m and above)																
Number of animal at risk				26	22	19	14	16	9	10	9	12	18	18	15	10
Number of animals positive				4	4	3	1	7	3	2	0	1	3	8	4	2
Percentage positive				15.4	18.2	15.8	7.1	43.8	33.3	20.0	0.0	8.3	16.7	44.4	26.7	20.0

- = Animals not available for sampling. While calculating animals at the risk, those animals were also considered which were found negative for three months after the anthelmintic treatment.

Appendix Table 4.4.19
Longitudinal Survey of Farms/Flocks (1991-1992)
Monthly faecal *F. gigantica* egg counts of tracer calves (cattle) in the Terai

Description of animal			Farm location	EPG during different months												
No	Age	Sex	VDC area	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	1.5	F	Tarahara	0.0	0.0	-	0.0	0.0	0.0	0.0	-	-	-	-	-	-
2	2.0	M	Tarahara	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0
3	1.5	F	Tarahara	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	-	-
4	1.5	M	Tarahara	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
5	1.0	M	Tarahara	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-
6	1.5	F	Tarahara	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-
7	0.5	M	Tarahara	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-
8	1.0	M	Tarahara	0.0	0.0	0.0	2.6	1.3	0.0	0.0	-	-	-	-	-	-
9	1.5	F	Tarahara	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	1.5	M	Tarahara	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	0.5	F	Tarahara	0.0	0.0	0.0	3.9	2.6	0.0	0.0	0.0	-	-	-	-	-
12	0.5	F	Tarahara	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-
13	1.0	F	Tarahara	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0
14	1.5	M	Tarahara	0.0	0.0	0.0	2.6	1.3	0.0	0.0	-	-	-	-	-	-
15	0.4	M	Tarahara	0.0	0.0	-	0.0	0.0	0.0	0.0	-	-	-	-	-	-
16	2.0	M	Tarahara	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	1.5	F	Tarahara	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	-	-
18	1.0	F	Tarahara	0.0	0.0	0.0	3.9	2.6	0.0	0.0	0.0	-	-	-	-	-
19	0.5	F	Tarahara	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
20	1.5	M	Tarahara	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.5	M	Tarahara	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-
22	0.5	M	Tarahara	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-
23	2.0	F	Letang	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.2	0.0	1.3	0.0
24	1.5	F	Letang	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0
25	0.4	F	Letang	0.0	2.6	0.0	1.3	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
26	1.5	M	Letang	0.0	0.0	1.3	0.0	1.3	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0
27	1.0	F	Letang	0.0	0.0	0.0	0.0	0.0	0.0	1.3	-	0.0	1.3	-	-	-
28	2.0	M	Letang	0.0	0.0	0.0	1.3	1.3	13.0	3.9	1.3	0.0	0.0	0.0	0.0	0.0
29	1.5	M	Letang	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-
30	1.5	F	Letang	0.0	0.0	0.0	0.0	2.6	0.0	1.3	0.0	0.0	0.0	0.0	3.9	0.0
31	1.0	M	Letang	0.0	0.0	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	6.5	-
32	0.4	M	Letang	0.0	-	1.3	6.5	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33	1.0	F	Letang	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-
34	0.5	M	Letang	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0
35	1.0	F	Letang	0.0	0.0	0.0	1.3	1.3	10.4	6.5	1.3	0.0	0.0	0.0	0.0	0.0
36	0.4	F	Letang	0.0	0.0	0.0	1.3	1.3	6.5	5.2	1.3	0.0	0.0	0.0	6.5	0.0
37	1.0	F	Letang	0.0	0.0	0.0	0.0	0.0	0.0	1.3	-	0.0	1.3	-	-	-
38	1.5	M	Letang	0.0	2.6	0.0	1.3	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
39	2.0	M	Letang	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.2	0.0	1.3	0.0
40	1.5	M	Letang	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	-	-	-	-
41	0.4	F	Letang	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-
42	0.5	M	Letang	0.0	0.0	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
43	1.0	F	Letang	0.0	0.0	0.0	0.0	2.6	0.0	1.3	0.0	0.0	0.0	0.0	3.9	0.0
44	1.5	F	Letang	0.0	0.0	1.3	0.0	1.3	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0
45	1.0	F	Jumka/Bhanta	0.0	0.0	0.0	9.1	0.0	5.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
46	1.0	M	Jumka/Bhanta	1.3	0.0	1.3	6.5	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
47	2.0	F	Jumka/Bhanta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-
48	0.4	M	Jumka/Bhanta	0.0	1.3	0.0	6.5	0.0	-	0.0	-	0.0	0.0	0.0	-	0.0
49	1.0	F	Jumka/Bhanta	0.0	0.0	0.0	0.0	0.0	0.0	1.3	-	0.0	0.0	-	-	-
50	1.0	M	Jumka/Bhanta	2.6	0.0	0.0	5.2	0.0	-	0.0	0.0	0.0	-	2.6	3.9	-
51	2.0	M	Jumka Bhanta	0.0	0.0	0.0	2.6	0.0	2.6	-	1.3	0.0	0.0	0.0	0.0	0.0
52	1.5	F	Jumka Bhanta	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	1.3	0.0	0.0
53	2.0	M	Jumka/Bhanta	0.0	0.0	0.0	0.0	0.0	0.0	1.3	-	0.0	0.0	-	-	-
54	1.5	M	Jumka Bhanta	1.3	0.0	1.3	5.2	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
55	0.5	F	Jumka Bhanta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0
56	1.5	M	Jumka Bhanta	0.0	0.0	0.0	9.1	0.0	5.2	0.0	0.0	0.0	0.0	1.3	1.3	0.0
57	0.4	F	Jumka Bhanta	0.0	1.3	0.0	3.9	0.0	-	0.0	-	0.0	0.0	0.0	-	0.0
58	0.5	F	Jumka Bhanta	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
59	2.0	F	Jumka Bhanta	3.9	0.0	0.0	7.8	0.0	-	0.0	0.0	0.0	-	2.6	1.3	-
60	1.5	M	Jumka Bhanta	0.0	0.0	0.0	2.6	0.0	2.6	-	1.3	0.0	0.0	0.0	0.0	0.0
OVERALL																
Number of animal at risk				60	55	50	49	32	27	24	17	23	27	25	23	19
Number of animals positive				4	4	3	17	5	2	8	0	0	5	3	5	0
Percentage positive				6.7	7.3	6.0	34.7	15.6	7.4	33.3	0.0	0.0	18.5	12.0	21.7	0.0
TARAHARA																
Number of animal at risk				22	22	20	22	16	15	13	8	6	6	6	6	6
Number of animals positive				0	0	0	6	1	0	4	0	0	0	0	0	0
Percentage positive				0.0	0.0	0.0	27.3	6.3	0.0	30.8	0.0	0.0	0.0	0.0	0.0	0.0
LETANG																
Number of animal at risk				22	21	20	17	10	6	6	8	11	12	8	10	5
Number of animals positive				0	2	3	7	4	0	2	0	0	5	0	4	0
Percentage positive				0.0	9.5	15.0	41.2	40.0	0.0	33.3	0.0	0.0	41.7	0.0	40.0	0.0
JHUMKA/BHANTABARI																
Number of animal at risk				16	12	10	10	6	6	5	1	6	9	11	7	8
Number of animals positive				4	2	0	4	0	2	2	0	0	0	3	1	0
Percentage positive				25.0	16.7	0.0	40.0	0.0	33.3	40.0	0.0	0.0	0.0	27.3	14.3	0.0

- = Animals not available for sampling. While calculating animals at the risk, those animals were also considered wich were found negative for three months after the anthelmintic treatment.

Appendix Table 4.4.20
Longitudinal Survey of Farm/Flock (1991-1992)
Monthly faecal *F. gigantica* egg counts of tracer buffalo calves in the hills

Description of animal			Farm location	EPG during different months												
No	Age	Sex	Altitude	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	2.0	F	600	1.3	22.1	0.0	5.2	6.5	0.0	0.0	3.9	1.3	0.0	7.8	0.0	0.0
2	1.5	M	600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
3	1.0	F	600	0.0	0.0	0.0	0.0	0.0	0.0	10.4	2.6	0.0	0.0	9.1	0.0	0.0
4	0.8	M	600	0.0	0.0	0.0	0.0	0.0	0.0	10.4	2.6	1.3	0.0	0.0	3.9	1.3
5	0.8	F	600	0.0	0.0	0.0	0.0	0.0	0.0	9.1	9.1	0.0	0.0	0.0	2.6	-
6	0.7	F	630	0.0	0.0	0.0	0.0	0.0	0.0	37.7	10.2	18.2	0.0	2.6	2.6	1.3
7	1.0	F	650	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-	0.0
8	2.0	M	650	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	-
9	1.0	M	650	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3		2.6	3.9	1.3	0.0
10	1.0	M	650	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	2.0	F	650	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-	0.0
12	2.0	M	700	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3		5.2	0.0	13.0	-
13	0.6	F	700	0.0	0.0	0.0	0.0	0.0	0.0	2.6	9.1	3.9	0.0	0.0	3.9	-
14	2.0	M	700	1.3	0.0	0.0	0.0	-	-	-	-	-	-	-	-	1.3
15	2.0	F	900	0.0	0.0	0.0	10.2	15.6	1.3	1.3	27.3	1.3	2.6	-	0.0	-
16	1.5	M	1150	7.8	0.0	0.0	-	-	-	-	-	-	-	-	-	-
17	1.5	M	1200	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-
18	1.5	M	1250	0.0	0.0	0.0	0.0	0.0	3.9	58.1	-	-	-	0.0	-	-
19	0.7	M	1300	0.0	0.0	0.0	0.0	0.0	0.0	18.2	2.6	2.6	-	-	-	-
20	2.0	F	1300	0.0	-	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	3.9	1.3	-
21	1.5	F	1300	0.0	1.3	0.0	-	-	-	-	-	-	-	-	-	-
22	2.0	F	1400	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	2.6	0.0	-
23	1.5	M	1400	-	-	-	-	-	-	-	-	-	-	-	-	-
24	2.0	F	1500	6.5	11.7	0.0	0.0	0.0	-	-	-	-	-	-	-	-
25	1.0	M	1500	0.0	2.6	0.0	6.5	5.2	0.0	3.9	0.0	0.0	0.0	0.0	2.6	14.3
26	0.7	M	1500	0.0	2.6	0.0	2.6	5.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1
27	0.7	M	1500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	1.3	-
28	2.0	F	1600	5.2	0.0	0.0	-	-	-	-	-	-	-	-	-	-
29	1.0	M	1700	1.3	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-
30	1.5	F	1700	2.6	0.0	0.0	0.0	-	-	-	-	-	-	-	-	-
31	2.0	F	1725	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	-	0.0	0.0	0.0	2.6
32	1.5	M	1750	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	5.2
33	1.5	M	1750	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0
34	2.0	F	1750	0.0	0.0	0.0	2.6	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
35	2.0	M	1750	0.0	-	-	-	0.0	0.0	-	-	-	-	-	-	-
OVERALL																
Number of animal at risk				34	25	23	23	21	21	16	9	8	9	10	6	10
Number of animals positive				7	3	0	2	0	2	7	5	1	1	3	1	4
Percentage positive				20.6	12.0	0.0	8.7	0.0	9.5	43.8	55.6	12.5	11.1	30.0	16.7	40.0
LOW ALTITUDE (below 1200 m)																
Number of animal at risk				16	13	13	13	10	10	10	5	2	1	1	1	6
Number of animals positive				3	0	0	1	0	0	5	3	1	0	0	0	1
Percentage positive				18.8	0.0	0.0	7.7	0.0	0.0	50.0	60.0	50.0	0.0	0.0	0.0	16.7
MID ALTITUDE (1200 m and above)																
Number of animal at risk				18	12	10	10	11	11	6	4	6	8	9	5	4
Number of animals positive				4	3	0	1	0	2	2	2	0	1	3	1	3
Percentage positive				22.2	25.0	0.0	10.0	0.0	18.2	33.3	50.0	0.0	12.5	33.3	20.0	75.0

- = Animals not available for sampling. While calculating animal at the risk those animals were also considered which were negative for three months after the anthelmintic treatment.

Appendix Table 4.4.21
Longitudinal Survey of Farm/Flock (1991-1992)
Monthly faecal *F. gigantica* egg counts of tracer buffalo calves in the Terai

Description of animal			Farm location	EPG during different months												
No	Age	Sex	VDC area	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	1.0	F	Tarahara	0.0	0.0	0.0	0.0	0.0	7.8	0.0	0.0	0.0	9.2	0.0	0.0	0.0
2	0.6	M	Tarahara	0.0	0.0	2.6	0.0	0.0	0.0	-	0.0	-	0.0	0.0	2.6	0.0
3	2.0	M	Tarahara	0.0	0.0	0.0	9.1	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.0
4	0.6	F	Tarahara	0.0	0.0	0.0	0.0	1.3	0.0	0.0	1.3	0.0	0.0	-	-	-
5	1.0	F	Tarahara	0.0	0.0	7.8	0.0	0.0	-	-	-	-	-	-	-	-
6	0.8	M	Tarahara	0.0	0.0	0.0	0.0	1.3	1.3	0.0	0.0	0.0	0.0	0.0	0.0	2.6
7	1.5	F	Tarahara	0.0	0.0	2.6	1.3	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0
8	2.0	F	Tarahara	6.5	0.0	0.0	0.0	0.0	2.6	1.3	0.0	0.0	0.0	0.0	0.0	0.0
9	2.0	M	Tarahara	0.0	0.0	1.3	0.0	0.0	0.0	-	-	-	-	-	-	0.0
10	0.6	M	Tarahara	0.0	0.0	0.0	0.0	5.2	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0
11	1.5	M	Tarahara	0.0	0.0	2.6	1.3	0.0	0.0	0.0	0.0		0.0	0.0	2.6	0.0
12	1.0	M	Tarahara	0.0	0.0	2.6	1.3	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0
13	0.5	F	Tarahara	0.0	0.0	0.0	0.0	5.2	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0
14	2.0	F	Tarahara	0.0	0.0	1.3	1.3	0.0	0.0	0.0	-	-	-	-	-	0.0
15	0.6	M	Tarahara	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	-	0.0	0.0	0.0	2.6
16	1.0	F	Tarahara	0.0	0.0	7.8	0.0	0.0	0.0	-	-	-	-	-	-	-
17	1.5	F	Tarahara	3.9	0.0	0.0	0.0	0.0	2.6	2.6	0.0	0.0	0.0	0.0	0.0	0.0
18	1.5	F	Tarahara	0.0	0.0	7.8	0.0	-	-	-	-	-	-	-	-	-
19	1.0	M	Tarahara	0.0	0.0	0.0	9.1	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.0
20	1.5	M	Tarahara	0.0	0.0	0.0	0.0	0.0	7.8	0.0	0.0	0.0	9.2	0.0	0.0	0.0
21	1.5	F	Letang	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0
22	0.8	M	Letang	0.0	1.3	0.0	0.0	0.0	0.0	-	-	0.0	5.2	0.0	0.0	0.0
23	0.6	F	Letang	0.0	14.3	0.0	0.0	0.0	1.3	0.0	0.0	1.3	0.0	0.0	0.0	-
24	0.8	F	Letang	2.6	0.0	1.3	0.0	0.0	-	-	-	-	-	-	-	-
25	0.4	M	Letang	0.0	0.0	6.5	0.0	0.0	-	-	-	-	-	-	-	-
26	0.7	M	Letang	0.0	0.0	0.0	1.3	0.0	0.0	0.0	-	-	-	-	-	-
27	0.6	M	Letang	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
28	0.5	M	Letang	0.0	6.5	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	0.0	0.0
29	0.6	F	Letang	1.3	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	-	-	-
30	0.5	F	Letang	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.0	0.0	0.0
31	1.0	M	Letang	0.0	14.3	0.0	0.0	0.0	0.0	0.0	0.0	-	2.6	0.0	0.0	0.0
32	1.0	M	Letang	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.4	0.0	-	-	-
33	1.5	M	Letang	0.0	1.3	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-
34	2.0	M	Letang	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	0.0	0.0
35	0.8	F	Letang	0.0	0.0	0.0	3.9	0.0	0.0	-	-	-	-	-	0.0	0.0
36	2.0	F	Letang	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.7	0.0
37	0.5	F	Letang	0.0	0.0	3.9	0.0	0.0	-	-	-	-	-	-	-	-
38	1.0	F	Letang	0.0	5.2	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0
39	0.5	F	Jhumka/Bhanta	0.0	0.0	0.0	10.4	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0
40	1.5	M	Jhumka/Bhanta	1.3	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0
41	1.0	M	Jhumka/Bhanta	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	3.9	0.0	0.0
42	1.5	M	Jhumka/Bhanta	1.3	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0	0.0	5.2	0.0	0.0
43	1.0	F	Jhumka/Bhanta	0.0	0.0	0.0	5.2	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0
44	1.7	F	Jhumka/Bhanta	0.0	0.0	2.6	0.0	0.0	0.0	0.0	-	10.8	0.0	0.0	0.0	-
45	0.6	M	Jhumka/Bhanta	0.0	3.9	0.0	0.0	0.0	-	-	-	-	-	-	-	-
46	1.0	F	Jhumka/Bhanta	0.0	0.0	0.0	0.0	0.0	0.0	13.0	0.0	0.0	-	-	-	-
47	2.0	F	Jhumka/Bhanta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	-	-
48	2.0	F	Jhumka/Bhanta	0.0	0.0	0.0	0.0	0.0	9.1	1.3	0.0	0.0	1.3	0.0	0.0	0.0
49	1.5	F	Jhumka/Bhanta	0.0	0.0	0.0	0.0	0.0	2.6	0.0	2.6	0.0	0.0	-	-	-
50	2.0	M	Jhumka/Bhanta	0.0	0.0	9.1	1.3	0.0	0.0	0.0	0.0	0.0	10.4	1.3	0.0	0.0
51	2.0	F	Jhumka/Bhanta	0.0	0.0	0.0	0.0	0.0	0.0	10.4	0.0	0.0	-	-	-	-
52	2.0	M	Jhumka/Bhanta	0.0	3.9	0.0	0.0	0.0	-	-	-	-	-	-	-	-
53	1.5	M	Jhumka/Bhanta	0.0	0.0	2.6	0.0	0.0	0.0	0.0		2.6	0.0	0.0	0.0	-
54	1.0	M	Jhumka/Bhanta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0
55	0.6	F	Jhumka/Bhanta	2.6	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-
56	0.7	M	Jhumka/Bhanta	0.0	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0
57	1.0	F	Jhumka/Bhanta	2.6	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-
58	0.5	M	Jhumka/Bhanta	0.0	0.0	2.6	1.3	0.0	0.0	0.0	0.0	0.0	10.4	2.6	1.3	0.0
OVERALL																
Number of animal at risk				58	49	40	26	22	24	17	23	21	25	24	15	13
Number of animals positive				9	9	14	9	5	7	3	2	3	5	10	4	4
Percentage positive				15.5	18.4	35.0	34.6	22.7	29.2	17.6	8.7	14.3	20.0	41.7	26.7	30.8
TARAHARA																
Number of animal at risk				20	18	18	9	8	4	0	4	4	8	12	10	10
Number of animals positive				2	0	9	2	5	4	0	0	0	0	2	2	4
Percentage positive				10.0	0.0	50.0	22.2	62.5	100.0	0.0	0.0	0.0	0.0	16.7	20.0	40.0
LETANG																
Number of animal at risk				18	15	8	6	5	11	9	9	7	9	6	3	2
Number of animals positive				3	7	2	3	0	1	1	0	1	3	3	1	0
Percentage positive				16.7	46.7	25.0	50.0	0.0	9.1	11.1	0.0	14.3	33.3	50.0	33.3	0.0
JHUMKA/BHANTABARI																
Number of animal at risk				20	16	14	11	9	9	8	10	10	8	6	2	1
Number of animals positive				4	2	3	4	0	2	2	2	2	2	5	1	0
Percentage positive				20.0	12.5	21.4	36.4	0.0	22.2	25.0	20.0	20.0	25.0	83.3	50.0	0.0

- = Animals not available for sampling. While calculating the animals at the risk, those animals were also considered which were found negative for three months after the anthelmintic treatment.

Appendix Table 4.5.1

Slaughter Place Survey: Buffaloes at Budhabare, Hile and Letang

Date	Number of animals		Details of the livers examined at the PAC laboratory										
			Description of animal				Fluke numbers			Fluke measurements (mm)			
	slaugh-tered	+ve	Age	Sex	Area	Liver wt. (kg)	Mature	Immature	Total	Length		Width	
										Range	Mean	Range	Mean
02-Jan-91	2	1	3.0	M	Mid	2.0	76	0	76	ND	ND	ND	ND
09-Jan-91	2	1	3.5	M	Low	4.0	146	0	146	ND	ND	ND	ND
10-Jan-91	4	3	8.0	F	Low	3.5	235	25	260	ND	ND	ND	ND
17-Jan-91	2	2	6.0	F	Mid	6.5	609	96	705	ND	ND	ND	ND
23-Jan-91	1	1	2.5	M	Mid	2.5	26	0	26	ND	ND	ND	ND
24-Jan-91	3	3	13.0	F	Mid	7.0	406	97	503	ND	ND	ND	ND
31-Jan-91	3	3	8.0	F	Low	4.0	283	0	283	ND	ND	ND	ND
07-Feb-91	3	3	4.0	F	High	3.3	550	0	550	ND	ND	ND	ND
11-Feb-91	2	2	1.0	M	Mid	1.1	2	0	2	ND	ND	ND	ND
11-Feb-91	2	2	1.3	M	Mid	1.6	68	0	68	ND	ND	ND	ND
14-Feb-91	4	3	8.0	F	High	4.0	190	0	190	ND	ND	ND	ND
21-Feb-91	4	3	8.0	F	Low	4.3	195	0	195	ND	ND	ND	ND
28-Feb-91	3	1	10.0	F	Mid	5.0	40	10	50	23-46	40.0	7-14	9.4
07-Mar-91	3	3	5.0	F	Mid	3.5	321	0	321	ND	ND	ND	ND
14-Mar-91	2	2	8.0	F	Mid	4.0	105	0	105	ND	ND	ND	ND
21-Mar-91	3	2	10.0	F	Mid	5.0	120	0	120	ND	ND	ND	ND
28-Mar-91	2	2	15.0	F	Low	6.5	385	0	385	ND	ND	ND	ND
04-Apr-91	2	1	8.0	F	Mid	5.0	235	0	235	ND	ND	ND	ND
11-Apr-91	2	1	6.0	F	Mid	3.5	117	0	117	35-50	43.5	9-12	10.5
18-Apr-91	3	3	12.0	F	Low	4.5	265	0	265	40-50	44.1	8-10	9.2
25-Apr-91	1	1	8.0	F	Mid	4.7	25	3	28	25-49	41.8	6-10	7.8
02-May-91	4	3	9.0	F	Low	4.8	115	0	115	32-50	44.1	10-14	11.6
09-May-91	3	2	6.0	F	Low	5.0	771	0	771	35-45	42.5	9-11	9.9
16-May-91	3	3	6.0	F	Mid	4.0	168	0	168	30-40	40.0	8-11	10.0
23-May-91	3	3	13.0	F	Mid	6.0	256	0	256	29-62	44.5	7-13	10.9
30-May-91	3	3	10.0	F	Low	5.5	270	4	274	23-55	44.3	7-14	10.4
06-Jun-91	4	4	6.0	M	Low	5.0	70	2	72	25-50	37.0	7-11	9.8
13-Jun-91	3	3	8.0	F	Low	6.0	110	0	110	30-40	33.1	4-9	6.8
27-Jun-91	4	4	8.0	F	Mid	4.5	240	0	240	32-50	42.3	5-12	9.6
04-Jul-91	4	2	9.0	F	Mid	12.5	509	278	787	13-43	28.8	2-10	5.5
11-Jul-91	1	1	20.0	F	Mid	8.0	985	51	1036	16-50	34.1	2-12	7.4
25-Jul-91	4	4	9.0	F	Mid	4.9	199	2	201	12-60	48.9	3-14	11.4
01-Aug-91	4	4	2.5	M	Mid	2.5	185	0	185	32-47	40.1	7-11	9.6
08-Aug-91	3	1	15.0	F	High	5.5	80	2	82	23-48	36.3	8-12	10.5
03-Aug-91	5	2	5.0	M	Terai	5.0	286	0	286	ND	ND	ND	ND
03-Aug-91	5	2	8.0	M	Terai	8.0	387	0	387	ND	ND	ND	ND
12-Aug-91	1	1	2.0	M	Mid	2.4	59	1	60	16-50	38.8	6-10	8.1
15-Aug-91	4	3	14.0	F	Low	5.0	380	0	380	30-45	37.1	7-14	9.7
22-Aug-91	5	4	7.0	F	Low	5.0	40	0	40	26-48	38.8	8-10	9.1
24-Aug-91	5	2	7.0	M	Terai	5.0	22	0	22	ND	ND	ND	ND
24-Aug-91	5	2	10.0	M	Terai	3.0	173	0	173	ND	ND	ND	ND
04-Sep-91	2	2	2.5	M	Mid	3.0	47	0	47	32-52	43.2	8-11	9.9
05-Sep-91	4	4	8.0	F	Mid	10.5	760	0	760	30-48	37.5	6-10	8.4
11-Sep-91	1	1	2.0	M	Mid	3.0	2	0	2	ND	ND	ND	ND
12-Sep-91	5	5	11.0	F	Low	5.0	195	0	195	32-44	37.4	8-10	10.8
18-Sep-91	1	1	2.0	M	Mid	2.0	1	0	1	ND	ND	ND	ND
19-Sep-91	3	3	13.0	F	Mid	6.5	152	0	152	32-55	41.8	7-12	9.3
21-Sep-91	7	6	9.0	M	Terai	5.0	335	0	335	ND	ND	ND	ND
21-Sep-91	7	6	10.0	M	Terai	6.0	430	0	430	ND	ND	ND	ND
21-Sep-91	7	6	10.0	M	Terai	4.0	300	0	300	ND	ND	ND	ND
21-Sep-91	7	6	10.0	M	Terai	5.0	250	0	250	ND	ND	ND	ND
21-Sep-91	7	6	6.0	M	Terai	3.0	500	0	500	ND	ND	ND	ND
21-Sep-91	7	6	1.5	M	Terai	2.0	30	0	30	ND	ND	ND	ND
25-Sep-91	3	3	3.0	M	Low	3.0	88	0	88	30-50	39.3	8-12	9.9
26-Sep-91	3	3	13.0	F	Mid	6.0	45	0	45	25-50	38.7	7-10	8.6
02-Oct-91	2	2	2.5	M	Mid	4.0	13	0	13	ND	ND	ND	ND
03-Oct-91	3	3	13.0	M	Mid	6.5	22	3	25	25-45	36.1	3-10	7.1
09-Oct-91	1	1	4.0	M	Low	4.0	144	0	144	30-48	39.4	9-11	10.2
29-Oct-91	3	2	15.0	M	Terai	6.0	30	0	30	ND	ND	ND	ND
29-Oct-91	3	2	15.0	M	Terai	5.0	130	0	130	ND	ND	ND	ND
30-Oct-91	3	2	5.0	M	Low	3.5	205	0	205	23-50	41.6	8-11	9.8
14-Nov-91	3	3	8.0	F	Low	6.0	510	0	510	22-48	35.2	6-10	9.0
21-Nov-91	4	4	8.0	F	Low	6.0	165	0	165	35-50	42.4	6-11	7.8
27-Nov-91	1	1	2.0	M	Low	2.0	45	0	45	32-48	38.4	7-10	9.3
28-Nov-91	4	4	13.0	F	Low	6.5	70	17	87	13-35	23.3	4-11	7.9
30-Nov-91	4	3	10.0	M	Terai	3.0	283	0	283	ND	ND	ND	ND
30-Nov-91	4	3	8.0	M	Terai	3.0	189	0	189	ND	ND	ND	ND
30-Nov-91	4	3	12.0	M	Terai	5.0	365	0	365	ND	ND	ND	ND
04-Dec-91	1	1	1.5	M	Low	2.0	125	3	128	25-42	35.1	8-11	9.7
05-Dec-91	4	3	8.0	F	Low	5.0	40	0	40	32-38	33.7	5-8	6.9
12-Dec-91	4	4	10.0	F	Mid	5.5	48	0	48	27-40	34.4	6-10	8.4
18-Dec-91	3	2	2.0	M	Low	2.0	55	0	55	35-50	43.5	9-11	10.0
19-Dec-91	3	3	13.0	F	Mid	4.3	140	0	140	35-58	46.5	7-14	10.1
21-Dec-91	3	1	10.0	F	Terai	3.0	397	0	397	ND	ND	ND	ND
25-Dec-91	2	2	2.0	F	Low	2.5	46	5	51	23-61	40.3	5-11	9.6
26-Dec-91	3	3	7.0	F	Mid	4.5	330	2	332	24-55	43.4	10-15	11.1
01-Jan-92	1	1	2.5	M	Mid	2.5	13	0	13	33-53	39.5	7-10	8.7
02-Jan-92	5	4	10.0	F	Low	6.0	215	0	215	30-58	43.5	8-15	11.5
08-Jan-92	2	1	2.0	M	Mid	2.5	26	4	30	14-53	41.0	3-14	10.3
09-Jan-92	2	2	10.0	F	Mid	5.0	135	20	155	17-41	31.4	3-13	10.1
16-Jan-92	1	1	11.0	F	Mid	11.8	71	2000	2071	4-43	21.3	1-10	3.6
22-Jan-92	1	1	2.0	M	Mid	1.5	4	0	4	28-50	41.8	9-10	9.8
22-Jan-92	5	4	10.0	M	Terai	3.0	212	0	212	ND	ND	ND	ND
22-Jan-92	5	4	10.0	M	Terai	3.0	393	0	393	ND	ND	ND	ND
22-Jan-92	5	4	10.0	M	Terai	3.0	184	0	184	ND	ND	ND	ND
22-Jan-92	5	4	10.0	M	Terai	2.0	94	0	94	ND	ND	ND	ND
23-Jan-92	5	5	8.0	F	Mid	8.5	280	100	380	22-50	34.5	3-11	6.9
28-Jan-92	1	1	2.5	M	Low	2.5	161	0	161	42-50	44.9	8-10	9.4

Note: ND = not determined

Continued

Appendix Table 4.5.1 (continued)

Date	Number of animals		Details of the liver examined at the PAC laboratory										
			Description of animal				Fluke counts			Fluke measurements (mm)			
	slaughtered	+ve	Age	Sex	Area	Liver wt. (kg)	Mature	Immature	Total	Length		Width	
										Range	Mean	Range	Mean
05-Feb-92	2	0	2.0	M	Mid	1.5	0	0	0	NA	NA	NA	NA
06-Feb-92	5	4	10.0	F	Low	5.0	370	8	378	24-54	42.0	8-13	10.7
12-Feb-92	2	1	2.5	M	Low	3.0	142	0	142	35-58	47.9	10-13	10.7
13-Feb-92	1	1	10.0	F	Mid	5.5	364	0	364	30-48	40.1	6-10	8.4
19-Feb-92	2	1	3.0	M	Mid	3.5	241	5	246	16-55	46.4	3-15	10.8
20-Feb-92	5	5	7.0	F	Low	5.0	81	0	81	26-42	34.9	6-10	8.4
26-Feb-92	1	1	2.0	M	Mid	2.0	60	0	60	35-60	48.4	9-14	11.6
27-Feb-92	3	3	6.0	M	Low	4.0	253	0	253	30-50	41.9	8-15	10.8
27-Feb-92	3	3	9.0	F	Mid	6.0	330	0	330	23-50	37.3	7-15	10.7
22-Feb-92	4	3	12.0	M	Terai	10.0	112	0	112	ND	ND	ND	ND
22-Feb-92	4	3	1.0	F	Terai	3.0	305	0	305	ND	ND	ND	ND
22-Feb-92	4	3	10.0	M	Terai	5.0	165	0	165	ND	ND	ND	ND
05-Mar-92	6	4	8.0	F	Low	4.5	0	0	0	NA	NA	NA	NA
12-Mar-92	4	4	5.0	F	Mid	6.0	187	3	190	23-50	43.2	7-10	8.3
12-Mar-92	4	4	4.0	F	Mid	5.0	160	50	210	7-50	37.5	1-11	7.5
19-Mar-92	6	6	2.5	M	Mid	2.5	195	0	195	36-52	42.2	7-10	8.6
19-Mar-92	6	6	17.0	F	Mid	5.0	420	0	420	27-47	39.0	7-10	8.3
19-Mar-92	6	6	14.0	F	High	11.0	390	80	470	15-41	32.6	3-10	6.7
25-Mar-92	3	2	2.0	M	Low	2.0	106	0	106	31-55	45.6	9-14	11.0
26-Mar-92	4	4	8.0	F	Mid	4.0	95	0	95	27-51	45.3	7-11	9.7
26-Mar-92	4	4	14.0	F	Mid	5.0	240	0	240	35-60	47.4	7-11	9.4
27-Mar-92	5	2	10.0	F	Terai	5.0	193	0	193	ND	ND	ND	ND
27-Mar-92	5	2	10.0	F	Terai	5.0	62	0	62	ND	ND	ND	ND
01-Apr-92	2	2	2.0	M	Low	2.0	130	0	130	28-52	42.6	9-13	10.4
02-Apr-92	4	2	13.0	F	Low	5.0	3	0	3	20-31	27.0	7-7	7.0
02-Apr-92	4	2	8.0	F	Low	4.0	80	0	80	30-50	38.5	6-10	8.5
08-Apr-92	2	2	0.8	M	Mid	2.0	3	15	18	5-40	22.9	1-8	4.4
09-Apr-92	4	2	8.0	F	Low	6.0	50	0	50	30-45	40.7	9-11	10.1
09-Apr-92	4	2	9.0	F	Low	9.0	75	0	75	35-55	43.7	10-12	10.3
15-Apr-92	2	2	1.5	M	Low	2.0	156	441	597	2-52	19.4	0.5-12	3.8
16-Apr-92	4	3	10.0	F	Low	5.0	296	0	296	20-45	29.3	6-10	8.3
16-Apr-92	4	3	5.0	F	Low	4.5	210	3	213	15-39	30.9	3-11	8.6
22-Apr-92	3	3	1.5	M	Low	1.5	15	0	15	30-40	32.7	6-11	9.2
23-Apr-92	6	3	2.0	M	Mid	5.0	1	6	7	10-35	18.0	3-7	4.8
23-Apr-92	6	3	18	F	Mid	7.5	220	17	237	25-50	40.6	6-14	10.6
23-Apr-92	6	3	7	F	Mid	6.0	256	0	256	30-50	38.6	6-11	8.6
29-Apr-92	2	2	2	M	Mid	2.0	35	0	35	27-45	39.5	6-11	9.9
30-Apr-92	5	5	8	F	Low	4.0	118	0	118	35-60	43.8	8-12	10.1
30-Apr-92	5	5	12	F	Mid	5.0	45	3	48	25-35	31.0	4-10	5.5
30-Apr-92	5	5	10	F	Mid	6.0	330	0	330	35-50	40.3	6-11	9.2
06-May-92	2	2	2	M	Low	2.0	55	1	56	10-55	44.2	4-14	10.7
07-May-92	3	3	10	F	Low	7.0	61	0	61	25-40	34.2	8-13	9.9
07-May-92	3	3	12	F	Low	5.0	226	1	227	12-45	36.4	4-12	9.5
07-May-92	3	3	18	F	Mid	8.0	278	0	278	26-45	37.1	6-13	8.8
02-May-92	5	3	10	F	Terai	3.0	124	0	124	ND	ND	ND	ND
02-May-92	5	3	10	F	Terai	5.0	226	0	226	ND	ND	ND	ND
02-May-92	5	3	10	F	Terai	4.0	305	0	305	ND	ND	ND	ND
13-May-92	2	1	2.5	M	Low	3.0	144	0	144	24-43	32.9	4-10	7.3
14-May-92	4	4	10	F	Low	7.0	215	0	215	26-42	35.8	6-11	9.4
14-May-92	4	4	11	F	Low	6.0	20	0	20	30-48	39.6	8-10	8.7
20-May-92	2	2	3	M	Mid	2.5	0	30	30	11-24	18.0	2-5	2.9
21-May-92	3	3	8	F	Low	5.0	160	0	160	27-50	41.2	9-15	11.6
21-May-92	3	3	8	F	Mid	4.0	13	0	13	35-46	39.8	7-10	9.4
16-May-92	5	2	11	F	Terai	5.0	356	0	356	ND	ND	ND	ND
16-May-92	5	2	7	F	Terai	5.0	230	0	230	ND	ND	ND	ND
27-May-92	1	1	2	M	Mid	2.5	24	0	24	28-48	40.9	5-12	10.2
28-May-92	2	2	9	F	Mid	6.0	324	0	324	20-40	35.3	9-13	10.5
28-May-92	2	2	10	F	Low	5.0	355	10	365	17-46	35.6	2-15	8.8
03-Jun-92	1	0	2	M	Mid	1.5	0	0	0	NA	NA	NA	NA
04-Jun-92	6	6	11	F	Low	8.0	46	0	46	35-50	42.2	7-12	9.4
04-Jun-92			6	F	Mid	6	172	0	172	35-48	39.0	5-11	10.2
10-Jun-92	1	1	2	M	Mid	2.5	11	0	11	30-55	46.0	10-10	10.0
11-Jun-92	6	5	12	F	Low	6.5	219	1	220	25-51	42.7	7-13	10.6
11-Jun-92			10	F	Low	8	448	4	452	25-40	33.5	6-11	9.01
17-Jun-92	1	0	2	M	Mid	2	0	0	0	NA	NA	NA	NA
18-Jun-92	6	5	2	M	Low	3	2	0	2	27-30	28.5	8-9	8.5
18-Jun-92			4	F	High	5	181	0	181	30-50	38.9	7-10	8.46
18-Jun-92			15	F	High	5	8	1	9	20-46	33.2	6-11	9.2
24-Jun-92	3	3	1.5	M	Mid	2	156	5	161	18-46	36.7	4-10	7.63
25-Jun-92	5	5	8	F	Low	7	6	0	6	35-41	37.0	8-10	9.4
25-Jun-92			11	F	Low	9	70	0	70	30-45	36.7	7-10	8.5
01-Jul-92	2	1	2	M	Low	2	0	0	0	NA	NA	NA	NA
02-Jul-92	5	5	10	F	Low	7	120	0	120	28-45	36.8	7-12	8.99
02-Jul-92			5	F	Mid	6	48	0	48	30-40	35.4	7-10	8.32
09-Jul-92	5	5	13	F	Mid	9	353	3	356	15-50	29.3	3-12	9.45
09-Jul-92			6	F	Low	8	30	0	30	30-46	39.0	9-10	9.62
16-Jul-92	7	4	8	F	Low	8.5	9	0	9	30-36	33.7	7-11	9.58
16-Jul-92			6	F	Low	11	90	21	111	15-45	29.3	3-10	6.5
23-Jul-92	4	2	13	F	Low	8.5	60	0	60	31-48	40.3	7-12	10.33
23-Jul-92			10	F	Low	9.5	65	0	65	30-41	34.7	7-10	9.1

Note: ND = not determined; NA = not applicable.

Appendix Table 8.1.1: Liveweights (kg) of sheep
Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

		Sheep given lower dose (150 metacercariae)						Sheep given higher dose (350 metacercariae)					
		F. gigantica		*F. hepatica*		Uninfected		*F. gigantica*		*F. hepatica*		Uninfected	
DATE	WPI	23	27	24	28	21	22	25	29	26	30	31	32
05-Apr-90	-4	44.0	46.0	41.0	53.0	46.0	53.0	22.0	35.0	22.0	34.0	39.0	23.0
12-Apr-90	-3	43.0	49.0	42.0	53.0	46.0	51.0	24.0	38.0	26.0	36.0	43.0	24.0
19-Apr-90	-2	42.0	48.0	41.0	51.0	46.5	50.5	23.0	39.0	26.5	35.0	41.0	25.5
26-Apr-90	-1	45.0	48.5	44.5	54.0	48.0	53.0	25.5	39.0	29.0	35.5	43.0	26.0
03-May-90	0	46.5	49.5	45.0	56.0	48.0	53.5	26.0	42.5	29.0	38.5	45.5	27.0
10-May-90	+1	49.5	51.0	47.5	56.5	51.0	56.0	29.5	43.0	31.0	38.0	47.5	29.0
17-May-90	+2	47.5	51.0	49.0	57.0	51.0	56.0	29.5	46.0	31.0	40.0	48.0	30.5
24-May-90	+3	46.5	51.0	49.5	57.5	50.0	57.0	30.0	44.5	34.0	38.5	49.0	29.0
31-May-90	+4	47.0	50.5	48.0	56.5	51.0	56.0	30.0	45.0	33.0	38.0	49.5	30.0
07-Jun-90	+5	46.0	44.5	46.0	50.5	48.0	50.0	29.0	45.0	31.0	35.0	44.0	29.0
14-Jun-90	+6	45.0	47.0	45.5	53.0	47.0	50.0	29.0	45.0	32.0	36.0	47.0	31.0
21-Jun-90	+7	45.0	47.0	46.0	54.0	47.0	50.0	33.0	46.0	33.0	37.0	46.0	29.0
28-Jun-90	+8	45.5	47.5	46.5	54.0	47.5	50.5	32.0	46.5	34.0	36.5	48.0	29.0
05-Jul-90	+9	45.0	47.0	47.0	52.0	47.0	52.0	31.0	48.0	31.5	36.0	48.0	30.0
12-Jul-90	+10	44.0	46.0	49.0	54.0	49.0	55.0	30.0	48.0	31.0	38.0	47.0	31.3
19-Jul-90	+11	41.5	44.0	45.5	51.0	51.0	51.0	31.5	41.0	30.0	36.0	45.5	32.0
26-Jul-90	+12	42.0	45.0	47.5	53.0	52.0	51.5	32.5	45.0	32.5	37.0	47.0	32.2
02-Aug-90	+13	42.0	43.0	50.0	56.0	52.5	54.0	30.0	49.0	31.5	37.5	49.0	33.5
09-Aug-90	+14	**	43.0	51.0	55.0	53.2	55.0	29.0	46.5	35.0	37.0	48.0	31.8
16-Aug-90	+15	**	45.0	51.0	56.0	53.0	56.5	**	47.5	32.0	39.5	51.0	33.8
23-Aug-90	+16	**	45.5	51.5	55.0	55.0	55.0	**	47.0	33.0	38.0	52.0	34.0
30-Aug-90	+17	**	45.0	50.0	54.0	54.8	58.0	**	47.0	31.5	38.5	50.0	35.0
06-Sep-90	+18	**	45.0	48.0	54.5	55.6	55.5	**	47.0	30.5	36.5	50.0	35.4
13-Sep-90	+19	**	46.0	50.5	55.5	56.0	57.5	**	46.0	32.0	40.0	50.0	34.5
20-Sep-90	+20	**	47.0	51.0	57.0	56.8	58.0	**	48.0	33.0	39.5	52.0	36.3

Note : ** = Sheep dead; all the sheep were sheared on 6th June 1990.

Appendix Table 8.1.2: *Fasciola* spp. faecal egg counts (EPG) of sheep
Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

		Sheep given lower dose (150 metacercariae)						Sheep given higher dose (350 metacercariae)					
		F. gigantica		*F. hepatica*		Uninfected		*F. gigantica*		*F. hepatica*		Uninfected	
DATE	WPI	23	27	24	28	21	22	25	29	26	30	31	32
05-Apr-90	-4	0	0	0	0	0	0	0	0	0	0	0	0
12-Apr-90	-3	0	0	0	0	0	0	0	0	0	0	0	0
19-Apr-90	-2	0	0	0	0	0	0	0	0	0	0	0	0
26-Apr-90	-1	0	0	0	0	0	0	0	0	0	0	0	0
03-May-90	0	0	0	0	0	0	0	0	0	0	0	0	0
10-May-90	+1	0	0	0	0	0	0	0	0	0	0	0	0
17-May-90	+2	0	0	0	0	0	0	0	0	0	0	0	0
24-May-90	+3	0	0	0	0	0	0	0	0	0	0	0	0
31-May-90	+4	0	0	0	0	0	0	0	0	0	0	0	0
07-Jun-90	+5	0	0	0	0	0	0	0	0	0	0	0	0
14-Jun-90	+6	0	0	0	0	0	0	0	0	0	0	0	0
21-Jun-90	+7	0	0	0	0	0	0	0	0	0	0	0	0
28-Jun-90	+8	0	0	0	0	0	0	0	0	0	0	0	0
05-Jul-90	+9	0	0	0	12	0	0	0	0	0	0	0	0
12-Jul-90	+10	0	0	15	117	0	0	0	0	0	12	0	0
19-Jul-90	+11	0	0	187	180	0	0	0	0	0	85	0	0
26-Jul-90	+12	0	0	69	66	0	0	0	0	1	13	0	0
02-Aug-90	+13	0	0	10	37	0	0	0	5	14	40	0	0
09-Aug-90	+14	20	59	41	4	0	0	0	29	39	53	0	0
16-Aug-90	+15	**	16	55	34	0	0	1	32	5	57	0	0
23-Aug-90	+16	**	36	20	41	0	0	**	7	48	116	0	0
30-Aug-90	+17	**	17	35	122	0	0	**	50	20	94	0	0
06-Sep-90	+18	**	55	37	59	0	0	**	32	118	105	0	0
13-Sep-90	+19	**	35	25	80	0	0	**	31	28	108	0	0
20-Sep-90	+20	**	30	26	72	0	0	**	51	258	112	0	0

Note : ** = Sheep dead.

Appendix Table 8.1.3: RBC counts ($\times 10^{12}$ cells/l) of sheep
Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

		Sheep given lower dose (150 metacercariae)						Sheep given higher dose (350 metacercariae)					
		F. gigantica		*F. hepatica*		Uninfected		*F. gigantica*		*F. hepatica*		Uninfected	
DATE	WPI	23	27	24	28	21	22	25	29	26	30	31	32
05-Apr-90	-4	13.5	14.1	13.8	13.1	13.7	14.2	13.5	11.8	15.8	14.5	11.3	12.3
12-Apr-90	-3	11.6	11.2	12.3	12.7	13.4	13.5	11.8	11.6	12.5	12.1	12.1	11.5
19-Apr-90	-2	13.0	14.2	13.7	13.0	14.2	14.1	11.3	13.9	14.1	13.4	14.5	14.8
26-Apr-90	-1	10.3	13.9	14.3	12.7	15.8	14.2	11.0	11.4	12.7	12.5	13.0	14.4
03-May-90	0	10.0	11.1	14.7	13.9	14.4	13.5	10.9	11.6	12.6	12.3	11.6	10.9
10-May-90	+1	10.5	12.0	12.9	11.4	13.8	14.7	10.8	12.1	12.9	12.7	11.0	12.5
17-May-90	+2	12.3	11.8	14.1	11.4	14.7	14.2	11.7	12.0	14.1	13.7	11.1	12.2
24-May-90	+3	11.3	12.4	14.9	12.5	15.2	13.9	11.2	12.7	13.3	13.8	11.8	12.2
31-May-90	+4	11.4	11.9	12.8	11.8	13.9	13.4	9.4	12.3	12.5	13.4	11.7	11.7
07-Jun-90	+5	10.6	11.6	13.7	11.5	14.1	13.5	9.1	12.2	11.9	12.3	11.2	11.1
14-Jun-90	+6	10.6	11.8	14.7	14.2	13.0	14.2	9.0	12.2	11.3	12.2	11.2	11.8
21-Jun-90	+7	11.2	12.2	14.2	13.4	14.3	14.6	8.4	13.2	11.3	13.0	11.8	12.1
28-Jun-90	+8	11.4	11.6	12.4	12.3	15.7	14.6	10.3	12.7	11.1	11.0	11.6	12.5
05-Jul-90	+9	11.0	12.6	12.1	12.0	14.1	14.1	9.3	13.1	11.4	10.4	11.8	12.5
12-Jul-90	+10	10.4	12.0	12.7	10.7	13.6	16.3	9.3	13.4	9.0	10.6	12.4	12.0
19-Jul-90	+11	10.3	11.6	11.7	9.6	15.7	14.3	8.4	12.2	8.3	9.1	11.3	12.1
26-Jul-90	+12	10.2	10.4	12.9	10.3	14.2	13.5	9.2	12.2	9.5	11.4	11.5	13.5
02-Aug-90	+13	7.4	6.1	10.6	10.2	12.6	11.6	6.7	10.4	7.3	7.9	11.6	12.0
09-Aug-90	+14	**	4.6	12.0	8.5	12.9	11.9	4.6	10.8	6.6	8.5	12.3	11.2
16-Aug-90	+15	**	6.5	11.4	10.1	13.5	12.6	**	11.8	6.1	10.3	12.4	12.2
23-Aug-90	+16	**	8.4	10.3	9.4	13.5	13.5	**	13.7	7.0	9.3	12.6	12.1
30-Aug-90	+17	**	7.2	9.7	8.2	12.9	12.5	**	10.5	6.1	8.2	12.7	11.9
06-Sep-90	+18	**	7.8	12.6	9.4	15.7	11.7	**	10.4	6.5	8.3	13.1	12.5
13-Sep-90	+19	**	7.6	10.5	9.9	12.4	12.1	**	10.5	6.3	6.8	11.9	12.1
20-Sep-90	+20	**	7.0	10.8	10.3	12.0	11.4	**	9.7	5.6	7.4	12.1	12.9

Note : ** = Sheep dead.

Appendix Table 8.1.4: Packed cell volumes (l/l) of sheep
Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

		Sheep given lower dose (150 metacercariae)						Sheep given higher dose (350 metacercariae)					
		F. gigantica		*F. hepatica*		Uninfected		*F. gigantica*		*F. hepatica*		Uninfected	
DATE	WPI	23	27	24	28	21	22	25	29	26	30	31	32
05-Apr-90	-4	0.30	0.32	0.33	0.29	0.32	0.34	0.28	0.30	0.31	0.28	0.30	0.31
12-Apr-90	-3	0.32	0.32	0.34	0.30	0.34	0.34	0.29	0.31	0.31	0.27	0.30	0.29
19-Apr-90	-2	0.31	0.32	0.35	0.30	0.34	0.34	0.31	0.32	0.31	0.32	0.33	0.31
26-Apr-90	-1	0.31	0.33	0.35	0.30	0.32	0.34	0.28	0.30	0.32	0.33	0.35	0.31
03-May-90	0	0.30	0.33	0.33	0.31	0.33	0.33	0.30	0.31	0.33	0.35	0.36	0.33
10-May-90	+1	0.31	0.33	0.36	0.29	0.34	0.35	0.29	0.30	0.34	0.34	0.34	0.32
17-May-90	+2	0.31	0.33	0.36	0.31	0.34	0.35	0.30	0.30	0.34	0.33	0.33	0.32
24-May-90	+3	0.32	0.35	0.36	0.32	0.34	0.34	0.32	0.31	0.34	0.35	0.35	0.33
31-May-90	+4	0.34	0.35	0.35	0.33	0.35	0.34	0.30	0.33	0.32	0.35	0.35	0.33
07-Jun-90	+5	0.30	0.31	0.35	0.29	0.33	0.34	0.28	0.30	0.31	0.31	0.35	0.31
14-Jun-90	+6	0.31	0.33	0.36	0.32	0.32	0.35	0.26	0.31	0.32	0.32	0.34	0.32
21-Jun-90	+7	0.31	0.33	0.36	0.33	0.35	0.33	0.26	0.31	0.31	0.32	0.35	0.34
28-Jun-90	+8	0.31	0.33	0.34	0.31	0.36	0.34	0.26	0.31	0.31	0.30	0.35	0.33
05-Jul-90	+9	0.31	0.33	0.28	0.30	0.32	0.32	0.27	0.29	0.28	0.28	0.34	0.34
12-Jul-90	+10	0.30	0.28	0.30	0.26	0.32	0.32	0.26	0.29	0.28	0.27	0.34	0.32
19-Jul-90	+11	0.29	0.28	0.28	0.25	0.34	0.33	0.26	0.31	0.23	0.24	0.33	0.33
26-Jul-90	+12	0.28	0.28	0.28	0.26	0.33	0.33	0.25	0.29	0.23	0.25	0.33	0.34
02-Aug-90	+13	0.22	0.17	0.28	0.23	0.33	0.32	0.22	0.26	0.21	0.23	0.33	0.32
09-Aug-90	+14	**	0.15	0.28	0.23	0.32	0.31	0.15	0.26	0.22	0.22	0.34	0.32
16-Aug-90	+15	**	0.20	0.27	0.26	0.32	0.32	0.12	0.26	0.23	0.26	0.34	0.32
23-Aug-90	+16	**	0.24	0.28	0.25	0.31	0.32	**	0.27	0.21	0.25	0.32	0.32
30-Aug-90	+17	**	0.22	0.27	0.23	0.31	0.30	**	0.26	0.21	0.26	0.32	0.34
06-Sep-90	+18	**	0.23	0.29	0.27	0.31	0.30	**	0.27	0.22	0.24	0.32	0.32
13-Sep-90	+19	**	0.24	0.31	0.30	0.30	0.32	**	0.27	0.22	0.23	0.33	0.33
20-Sep-90	+20	**	0.26	0.33	0.30	0.31	0.31	**	0.28	0.21	0.28	0.37	0.35

Note : ** = Sheep dead.

Appendix Table 8.1.5: Haemoglobin concentrations (g/l) of sheep
Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

		Sheep given lower dose (150 metacercariae)						Sheep given higher dose (350 metacercariae)					
		F. gigantica		*F. hepatica*		Uninfected		*F. gigantica*		*F. hepatica*		Uninfected	
DATE	WPI	23	27	24	28	21	22	25	29	26	30	31	32
05-Apr-90	-4	137	138	148	135	148	149	106	132	133	113	125	134
12-Apr-90	-3	141	138	148	132	148	142	104	132	129	113	134	119
19-Apr-90	-2	139	141	162	137	146	159	113	144	140	137	142	137
26-Apr-90	-1	138	148	158	133	144	150	103	132	134	142	152	138
03-May-90	0	125	144	148	133	142	137	103	137	141	152	153	141
10-May-90	+1	136	144	157	130	144	153	105	131	142	147	145	134
17-May-90	+2	137	147	157	136	145	153	114	132	142	146	145	136
24-May-90	+3	141	155	160	146	145	149	123	142	147	155	154	149
31-May-90	+4	140	148	156	139	146	144	101	139	144	151	150	145
07-Jun-90	+5	140	138	147	133	141	139	99	137	133	141	150	134
14-Jun-90	+6	135	145	167	141	136	150	91	134	140	137	149	137
21-Jun-90	+7	137	147	156	152	148	145	90	134	133	140	150	147
28-Jun-90	+8	140	142	137	125	141	140	96	139	118	118	145	146
05-Jul-90	+9	137	138	127	136	137	145	104	129	122	121	146	146
12-Jul-90	+10	130	128	132	116	132	131	95	133	100	114	141	131
19-Jul-90	+11	129	122	127	109	149	145	97	131	95	101	138	143
26-Jul-90	+12	124	119	121	113	140	139	92	124	99	102	142	146
02-Aug-90	+13	93	72	119	99	134	128	81	110	85	94	139	136
09-Aug-90	+14	**	59	122	101	127	135	50	116	84	110	146	136
16-Aug-90	+15	**	81	115	109	124	127	38	111	71	109	145	130
23-Aug-90	+16	**	105	123	114	121	142	**	114	84	108	140	125
30-Aug-90	+17	**	90	119	102	124	125	**	108	83	103	132	124
06-Sep-90	+18	**	93	126	109	122	130	**	114	85	98	139	125
13-Sep-90	+19	**	100	129	122	126	136	**	122	89	95	147	132
20-Sep-90	+20	**	108	149	136	128	140	**	119	88	112	163	156

Note : ** = Sheep dead.

Appendix Table 8.1.6: Mean cell volumes (fl) of sheep
Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

		Sheep given lower dose (150 metacercariae)						Sheep given higher dose (350 metacercariae)					
		F. gigantica		*F. hepatica*		Uninfected		*F. gigantica*		*F. hepatica*		Uninfected	
DATE	WPI	23	27	24	28	21	22	25	29	26	30	31	32
05-Apr-90	-4	22.2	22.7	23.9	22.1	23.4	23.9	20.7	25.4	19.6	19.3	26.5	25.2
12-Apr-90	-3	27.6	28.6	27.6	23.6	25.4	25.2	24.6	26.7	24.8	22.3	24.8	25.2
19-Apr-90	-2	23.8	22.5	25.5	23.1	23.9	24.1	27.4	23.0	22.0	23.9	22.8	20.9
26-Apr-90	-1	30.1	23.7	24.5	23.6	20.3	23.9	25.5	26.3	25.2	26.4	26.9	21.5
03-May-90	0	30.0	29.7	22.4	22.3	22.9	24.4	27.5	26.7	26.2	28.5	31.0	30.3
10-May-90	+1	29.5	27.5	27.9	25.4	24.6	23.8	26.9	24.8	26.4	26.8	30.9	25.6
17-May-90	+2	25.2	28.0	25.5	27.2	23.1	24.6	25.6	25.0	24.1	24.1	29.7	26.2
24-May-90	+3	28.3	28.2	24.2	25.6	22.4	24.5	28.6	24.4	25.6	25.4	29.7	27.0
31-May-90	+4	29.8	29.4	27.3	28.0	25.2	25.4	31.9	26.8	25.6	26.1	29.9	28.2
07-Jun-90	+5	28.3	26.7	25.5	25.2	23.4	25.2	30.8	24.6	26.1	25.2	31.3	27.9
14-Jun-90	+6	29.2	28.0	24.5	22.5	24.6	24.6	28.9	25.4	28.3	26.2	30.4	27.1
21-Jun-90	+7	27.7	27.0	25.4	24.6	24.5	22.6	31.0	23.5	27.4	24.6	29.7	28.1
28-Jun-90	+8	27.3	28.4	27.4	25.3	22.9	23.3	25.2	24.4	27.9	27.4	30.2	26.4
05-Jul-90	+9	28.3	26.2	23.1	25.0	22.8	22.8	29.0	22.1	24.6	27.1	28.8	27.2
12-Jul-90	+10	29.0	23.3	23.7	24.4	23.5	19.6	28.1	21.6	31.3	25.6	27.4	26.7
19-Jul-90	+11	28.3	24.2	24.0	26.0	21.7	23.2	31.0	25.5	27.7	26.4	29.2	27.3
26-Jul-90	+12	27.5	27.1	21.8	25.2	23.3	24.5	27.3	23.9	24.2	21.9	28.7	25.3
02-Aug-90	+13	29.9	28.1	26.4	22.5	26.3	27.6	32.8	25.1	28.8	29.1	28.6	26.7
09-Aug-90	+14	**	33.0	23.4	27.1	24.8	26.2	32.6	24.1	33.3	26.0	27.6	28.6
16-Aug-90	+15	**	30.8	23.8	25.7	23.7	25.4	**	22.0	37.7	25.2	27.4	26.2
23-Aug-90	+16	**	28.6	27.2	26.7	23.0	20.6	**	19.8	30.2	26.9	25.4	26.4
30-Aug-90	+17	**	30.6	28.0	28.2	24.1	24.0	**	24.9	34.7	30.0	25.3	28.7
06-Sep-90	+18	**	29.7	23.1	28.9	19.8	25.6	**	26.1	34.1	29.1	19.9	25.6
13-Sep-90	+19	**	31.8	29.5	30.5	24.2	26.6	**	25.8	34.9	33.8	27.9	27.4
20-Sep-90	+20	**	37.4	30.6	29.3	25.9	27.3	**	28.9	37.5	37.8	30.6	27.1

Note : ** = Sheep dead.

Appendix Table 8.1.7: Mean cell haemoglobin (pg) of sheep
Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

		Sheep given lower dose (150 metacercariae)						Sheep given higher dose (350 metacercariae)					
		F. gigantica		*F. hepatica*		Uninfected		*F. gigantica*		*F. hepatica*		Uninfected	
DATE	WPI	23	27	24	28	21	22	25	29	26	30	31	32
05-Apr-90	-4	10.1	9.8	10.7	10.3	10.8	10.5	7.9	11.2	8.4	7.8	11.1	10.9
12-Apr-90	-3	12.2	12.3	12.0	10.4	11.0	10.5	8.8	11.4	10.3	9.3	11.1	10.3
19-Apr-90	-2	10.7	9.9	11.8	10.5	10.3	11.3	10.0	10.4	9.9	10.2	9.8	9.3
26-Apr-90	-1	13.4	10.6	11.0	10.5	9.1	10.6	9.4	11.6	10.6	11.4	11.7	9.6
03-May-90	0	12.5	13.0	10.1	9.6	9.9	10.1	9.4	11.8	11.2	12.4	13.2	12.9
10-May-90	+1	13.0	12.0	12.2	11.4	10.4	10.4	9.7	10.8	11.0	11.6	13.2	10.7
17-May-90	+2	11.1	12.5	11.1	11.9	9.9	10.8	9.7	11.0	10.1	10.7	13.1	11.1
24-May-90	+3	12.5	12.5	10.7	11.7	9.5	10.7	11.0	11.2	11.1	11.2	13.1	12.2
31-May-90	+4	12.3	12.4	12.2	11.8	10.5	10.7	10.7	11.3	11.5	11.3	12.8	12.4
07-Jun-90	+5	13.2	11.9	10.7	11.6	10.0	10.3	10.9	11.2	11.2	11.5	13.4	12.1
14-Jun-90	+6	12.7	12.3	11.4	9.9	10.5	10.6	10.1	11.0	12.4	11.2	13.3	11.6
21-Jun-90	+7	12.2	12.0	11.0	11.3	10.3	9.9	10.7	10.2	11.8	10.8	12.7	12.1
28-Jun-90	+8	12.3	12.2	11.0	10.2	9.0	9.6	9.3	10.9	10.6	10.8	12.5	11.7
05-Jul-90	+9	12.5	11.0	10.5	11.3	9.8	10.3	11.2	9.8	10.7	11.7	12.4	11.7
12-Jul-90	+10	12.6	10.7	10.4	10.9	9.7	8.0	10.3	9.9	11.2	10.8	11.4	10.9
19-Jul-90	+11	12.6	10.6	10.9	11.4	9.5	10.2	11.5	10.8	11.4	11.1	12.2	11.8
26-Jul-90	+12	12.2	11.5	9.4	11.0	9.9	10.3	10.1	10.2	10.4	8.9	12.3	10.9
02-Aug-90	+13	12.7	11.9	11.2	9.7	10.7	11.0	12.1	10.6	11.6	11.9	12.0	11.3
09-Aug-90	+14	**	13.0	10.2	11.9	9.8	11.4	10.9	10.7	12.7	13.0	11.9	12.1
16-Aug-90	+15	**	12.5	10.1	10.8	9.2	10.1	12.0	9.4	11.6	10.6	11.7	10.7
23-Aug-90	+16	**	12.5	11.9	12.2	9.0	9.2	**	8.4	12.1	11.6	11.1	10.3
30-Aug-90	+17	**	12.5	12.3	12.5	9.6	10.0	**	10.3	13.7	12.6	10.4	10.5
06-Sep-90	+18	**	12.0	10.0	11.7	7.8	11.1	**	11.0	13.2	11.9	8.6	10.0
13-Sep-90	+19	**	13.2	12.3	12.4	10.2	11.3	**	11.7	14.1	14.0	12.4	11.0
20-Sep-90	+20	**	15.5	13.8	13.3	10.7	12.3	**	12.3	15.7	15.1	13.5	12.1

Note : ** = Sheep dead.

Appendix Table 8.1.8: Mean cell haemoglobin concentrations (g/l) of sheep
Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

		Sheep given lower dose (150 metacercariae)						Sheep given higher dose (350 metacercariae)					
		F. gigantica		*F. hepatica*		Uninfected		*F. gigantica*		*F. hepatica*		Uninfected	
DATE	WPI	23	27	24	28	21	22	25	29	26	30	31	32
05-Apr-90	-4	456.7	431.3	448.5	465.5	462.5	438.2	378.6	440.0	429.0	403.6	416.7	432.3
12-Apr-90	-3	440.6	431.3	435.3	440.0	435.3	417.6	358.6	425.8	416.1	418.5	446.7	410.3
19-Apr-90	-2	448.4	440.6	462.9	456.7	429.4	467.6	364.5	450.0	451.6	428.1	430.3	441.9
26-Apr-90	-1	445.2	448.5	451.4	443.3	450.0	441.2	367.9	440.0	418.8	430.3	434.3	445.2
03-May-90	0	416.7	436.4	448.5	429.0	430.3	415.2	343.3	441.9	427.3	434.3	425.0	427.3
10-May-90	+1	438.7	436.4	436.1	448.3	423.5	437.1	362.1	436.7	417.6	432.4	426.5	418.8
17-May-90	+2	441.9	445.5	436.1	438.7	426.5	437.1	380.0	440.0	417.6	442.4	439.4	425.0
24-May-90	+3	440.6	442.9	444.4	456.3	426.5	438.2	384.4	458.1	432.4	442.9	440.0	451.5
31-May-90	+4	411.8	422.9	445.7	421.2	417.1	423.5	336.7	421.2	450.0	431.4	428.6	439.4
07-Jun-90	+5	466.7	445.2	420.0	458.6	427.3	408.8	353.6	456.7	429.0	454.8	428.6	432.3
14-Jun-90	+6	435.5	439.4	463.9	440.6	425.0	428.6	350.0	432.3	437.5	428.1	438.2	428.1
21-Jun-90	+7	441.9	445.5	433.3	460.6	422.9	439.4	346.2	432.3	429.0	437.5	428.6	432.4
28-Jun-90	+8	451.6	430.3	402.9	403.2	391.7	411.8	369.2	448.4	380.6	393.3	414.3	442.4
05-Jul-90	+9	441.9	418.2	453.6	453.3	428.1	453.1	385.2	444.8	435.7	432.1	429.4	429.4
12-Jul-90	+10	433.3	457.1	440.0	446.2	412.5	409.4	365.4	458.6	357.1	422.2	414.7	409.4
19-Jul-90	+11	444.8	435.7	453.6	436.0	438.2	439.4	373.1	422.6	413.0	420.8	418.2	433.3
26-Jul-90	+12	442.9	425.0	432.1	434.6	424.2	421.2	368.0	427.6	430.4	408.0	430.3	429.4
02-Aug-90	+13	422.7	423.5	425.0	430.4	406.1	400.0	368.2	423.1	404.8	408.7	421.2	425.0
09-Aug-90	+14	**	393.3	435.7	439.1	396.9	435.5	333.3	446.2	381.8	450.0	429.4	425.0
16-Aug-90	+15	**	405.0	425.9	419.2	387.5	396.9	318.0	426.9	358.7	419.2	426.5	406.3
23-Aug-90	+16	**	437.5	439.3	456.0	390.3	443.8	**	422.2	400.0	432.0	437.5	390.6
30-Aug-90	+17	**	409.1	440.7	443.5	400.0	416.7	**	415.4	395.2	396.2	412.5	364.7
06-Sep-90	+18	**	404.3	434.5	403.7	393.5	433.3	**	422.2	386.4	408.3	434.4	390.6
13-Sep-90	+19	**	416.7	416.1	406.7	420.0	425.0	**	451.9	404.5	413.0	445.5	400.0
20-Sep-90	+20	**	415.4	451.5	453.3	412.9	451.6	**	425.0	419.0	400.0	440.5	445.7

Note : ** = Sheep dead.

Appendix Table 8.1.9: Total WBC counts (x10^9 cells/l) of sheep
Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

		Sheep given lower dose (150 metacercariae)						Sheep given higher dose (350 metacercariae)					
		F. gigantica		*F. hepatica*		Uninfected		*F. gigantica*		*F. hepatica*		Uninfected	
DATE	WPI	23	27	24	28	21	22	25	29	26	30	31	32
05-Apr-90	-4	11.5	7.8	6.8	7.8	16.3	20.2	9.0	7.4	11.5	7.8	13.7	8.0
12-Apr-90	-3	16.4	9.3	11.6	10.7	16.4	14.3	8.9	12.5	10.0	10.8	8.2	9.5
19-Apr-90	-2	11.9	7.8	13.2	8.3	14.9	16.5	10.6	11.0	9.3	7.0	7.6	9.2
26-Apr-90	-1	15.6	10.7	18.6	10.7	21.4	16.3	12.7	13.0	9.8	8.0	10.4	10.9
03-May-90	0	7.6	7.0	11.3	7.1	11.7	12.7	8.7	11.7	8.2	5.5	9.2	10.3
10-May-90	+1	10.8	9.1	14.8	5.9	17.1	10.8	10.7	8.6	9.3	7.6	9.0	10.3
17-May-90	+2	15.3	12.7	15.5	10.4	18.0	19.8	16.5	12.3	14.7	9.6	9.6	9.5
24-May-90	+3	16.9	15.5	19.6	18.5	15.4	16.8	25.7	15.4	16.3	14.2	12.1	11.8
31-May-90	+4	14.8	11.6	13.6	11.5	14.0	16.6	23.7	13.2	14.8	16.6	10.6	11.3
07-Jun-90	+5	19.4	14.7	20.7	14.2	16.7	17.5	28.4	14.2	17.8	16.3	15.3	12.7
14-Jun-90	+6	16.6	13.1	19.5	14.6	18.1	19.8	25.9	13.7	14.2	12.8	12.1	12.3
21-Jun-90	+7	15.4	13.4	20.1	20.9	20.4	19.2	21.6	14.4	18.6	15.6	13.7	12.2
28-Jun-90	+8	16.7	16.1	12.9	13.9	15.1	19.2	17.9	14.2	11.8	14.7	16.3	14.6
05-Jul-90	+9	15.2	16.8	18.1	18.6	17.0	18.6	20.8	10.1	14.3	13.9	14.7	14.4
12-Jul-90	+10	17.2	8.2	14.9	18.1	20.1	19.3	16.2	12.3	16.6	13.2	11.0	12.1
19-Jul-90	+11	12.5	8.1	14.2	11.2	18.0	18.0	14.6	11.1	13.3	11.0	10.1	11.6
26-Jul-90	+12	12.1	8.5	13.7	12.5	16.3	15.4	13.9	25.0	14.4	9.6	14.2	11.1
02-Aug-90	+13	9.5	8.9	14.4	8.6	11.5	12.9	14.7	15.1	12.4	9.0	10.4	10.9
09-Aug-90	+14	**	6.6	10.6	6.5	10.5	13.8	12.1	16.3	9.3	6.4	10.1	11.3
16-Aug-90	+15	**	6.5	10.7	10.1	11.6	12.8	**	11.8	6.1	10.3	12.3	12.8
23-Aug-90	+16	**	6.5	10.5	8.4	10.4	14.9	**	8.9	11.0	6.1	8.9	9.4
30-Aug-90	+17	**	6.5	9.6	6.6	11.4	11.5	**	10.4	6.9	6.1	9.2	10.0
06-Sep-90	+18	**	4.7	7.6	6.1	12.1	16.5	**	7.8	6.2	5.8	7.8	9.6
13-Sep-90	+19	**	5.4	9.1	6.9	9.3	13.0	**	8.0	8.7	5.1	8.0	8.6
20-Sep-90	+20	**	6.7	12.1	9.2	9.7	13.1	**	9.8	8.2	9.7	11.2	11.7

Note : ** = Sheep dead.

Appendix Table 8.1.10: Eosinophil counts (x10^9 cells/l) of sheep
Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

		Sheep given lower dose (150 metacercariae)						Sheep given higher dose (350 metacercariae)					
		F. gigantica		*F. hepatica*		Uninfected		*F. gigantica*		*F. hepatica*		Uninfected	
DATE	WPI	23	27	24	28	21	22	25	29	26	30	31	32
05-Apr-90	-4	0.07	0.29	0.27	0.07	0.53	0.45	0.48	0.15	0.38	0.06	0.07	0.06
12-Apr-90	-3	0.08	0.32	0.36	0.13	0.51	0.42	0.54	0.19	0.13	0.10	0.08	0.18
19-Apr-90	-2	0.08	0.19	0.43	0.13	0.57	0.47	0.57	0.18	0.13	0.15	0.06	0.08
26-Apr-90	-1	0.09	0.17	0.32	0.06	0.40	0.33	0.49	0.16	0.10	0.25	0.11	0.16
03-May-90	0	0.06	0.09	0.22	0.05	0.38	0.20	0.27	0.12	0.06	0.11	0.08	0.05
10-May-90	+1	0.09	0.33	0.55	0.09	0.33	0.18	0.32	0.13	0.32	0.24	0.08	0.04
17-May-90	+2	1.11	2.18	1.82	0.83	0.36	0.19	3.84	0.88	2.73	1.65	0.07	0.05
24-May-90	+3	2.01	2.30	2.28	1.06	0.34	0.10	5.80	1.59	3.36	2.62	0.04	0.07
31-May-90	+4	1.75	0.98	1.50	0.83	0.43	0.18	6.83	0.72	3.04	3.15	0.04	0.04
07-Jun-90	+5	1.98	1.16	0.97	0.69	0.42	0.18	6.22	0.33	2.25	3.43	0.04	0.03
14-Jun-90	+6	0.78	0.79	0.71	0.53	0.19	0.09	5.98	0.24	1.35	2.13	0.05	0.03
21-Jun-90	+7	1.03	1.46	1.66	1.05	0.27	0.10	2.73	0.35	2.46	2.60	0.16	0.04
28-Jun-90	+8	0.93	1.35	1.49	1.26	0.33	0.14	1.67	0.51	2.18	2.06	0.05	0.03
05-Jul-90	+9	0.34	1.33	2.08	2.32	0.44	0.08	2.27	0.48	3.15	3.13	0.08	0.05
12-Jul-90	+10	0.48	0.83	2.03	2.25	0.21	0.08	2.09	0.69	4.28	3.02	0.09	0.06
19-Jul-90	+11	0.88	1.19	1.47	1.09	0.35	0.09	2.28	1.76	3.58	2.38	0.09	0.07
26-Jul-90	+12	0.90	1.49	2.01	0.60	0.67	0.11	2.53	4.61	3.20	1.97	0.04	0.03
02-Aug-90	+13	1.45	1.54	1.94	0.18	0.21	0.08	3.24	2.48	3.21	1.49	0.05	0.04
09-Aug-90	+14	**	0.83	0.80	0.10	0.26	0.07	1.90	0.50	1.83	0.65	0.05	0.05
16-Aug-90	+15	**	0.27	0.45	0.09	0.23	0.07	**	0.17	1.95	0.48	0.04	0.03
23-Aug-90	+16	**	0.37	0.37	0.03	0.04	0.04	**	0.10	2.45	0.45	0.03	0.03
30-Aug-90	+17	**	0.62	0.38	0.07	0.12	0.07	**	0.18	1.20	0.67	0.04	0.03
06-Sep-90	+18	**	0.23	0.23	0.12	0.29	0.16	**	0.14	0.56	0.84	0.05	0.04
13-Sep-90	+19	**	0.06	0.20	0.04	0.19	0.06	**	0.12	1.40	0.38	0.06	0.06
20-Sep-90	+20	**	0.10	0.17	0.09	0.23	0.05	**	0.09	0.78	0.23	0.04	0.04

Note : ** = Sheep dead.

Appendix Table 8.1.11: Serum glutamate dehydrogenase activities (U/l) of sheep
Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

		Sheep given lower dose (150 metacercariae)						Sheep given higher dose (350 metacercariae)					
		F. gigantica		*F. hepatica*		Uninfected		*F. gigantica*		*F. hepatica*		Uninfected	
DATE	WPI	23	27	24	28	21	22	25	29	26	30	31	32
05-Apr-90	-4	5.8	3.0	4.2	2.4	3.4	5.7	3.5	3.4	3.2	2.8	3.5	3.2
12-Apr-90	-3	8.5	1.8	2.8	2.8	2.2	5.5	2.4	3.3	3.0	1.4	3.3	2.2
19-Apr-90	-2	7.7	2.5	3.7	3.5	2.9	5.5	2.2	3.9	3.9	2.0	3.5	3.3
26-Apr-90	-1	5.1	1.2	2.0	2.8	2.2	2.6	3.2	3.9	4.9	2.4	3.0	2.0
03-May-90	0	7.9	3.0	2.0	2.8	4.5	3.3	2.4	3.3	5.7	2.2	3.0	2.6
10-May-90	+1	10.6	4.7	4.9	8.1	4.3	5.9	4.9	7.1	15.6	3.9	3.8	4.7
17-May-90	+2	27.6	33.1	46.5	9.7	1.6	9.7	88.5	13.2	85.7	61.1	3.9	2.4
24-May-90	+3	139.9	88.3	100.5	47.3	5.5	7.7	341.8	42.4	301.0	253.2	4.3	3.4
31-May-90	+4	317.3	88.7	77.8	39.4	3.4	5.7	357.6	56.2	295.5	396.0	5.9	3.2
07-Jun-90	+5	120.2	46.3	54.2	38.4	4.7	7.7	375.3	31.5	199.0	324.1	7.2	3.0
14-Jun-90	+6	112.3	45.3	69.9	29.6	2.2	6.5	157.6	15.8	114.3	126.1	3.4	3.2
21-Jun-90	+7	113.5	68.0	109.4	64.0	3.2	5.3	149.7	23.3	140.9	146.8	4.5	3.6
28-Jun-90	+8	53.2	36.5	171.4	97.5	2.6	5.3	37.4	30.9	135.0	139.9	2.6	2.8
05-Jul-90	+9	64.0	41.4	189.1	140.9	3.0	4.9	60.1	49.3	179.3	115.2	3.0	3.9
12-Jul-90	+10	55.2	32.5	202.9	105.4	3.9	4.9	50.2	79.8	202.9	122.1	4.9	4.9
19-Jul-90	+11	84.7	57.1	169.4	40.4	2.6	8.9	68.0	240.3	154.7	44.5	4.3	3.2
26-Jul-90	+12	113.3	72.9	92.6	10.8	3.5	8.7	63.0	375.3	102.4	38.4	5.7	1.8
02-Aug-90	+13	159.6	46.3	93.2	5.9	2.6	8.3	69.9	207.8	69.9	20.7	3.9	2.2
09-Aug-90	+14	**	23.6	45.3	9.9	1.8	9.7	87.7	38.4	76.8	34.5	4.6	1.2
16-Aug-90	+15	**	8.9	29.6	7.9	3.8	7.8	96.5	14.8	81.2	14.8	4.2	1.3
23-Aug-90	+16	**	7.9	32.9	4.7	2.4	3.2	**	9.9	53.8	49.8	2.8	1.8
30-Aug-90	+17	**	3.7	27.8	3.3	2.0	3.3	**	16.2	21.9	90.2	5.1	2.0
06-Sep-90	+18	**	3.3	44.9	8.9	1.2	4.9	**	13.2	43.7	66.0	3.5	2.2
13-Sep-90	+19	**	2.9	16.9	4.3	1.8	5.7	**	7.5	37.4	18.9	7.7	2.6
20-Sep-90	+20	**	3.0	35.1	6.3	1.4	5.3	**	7.7	20.9	10.1	4.9	1.8

Note : ** = Sheep dead.

Appendix Table 8.1.12: Serum gamma glutamyl transpeptidase activities (U/l) of sheep
Experiment 1: Comparative pathogenicity of *F. gigantica* and *F. hepatica* in sheep

		Sheep given lower dose (150 metacercariae)						Sheep given higher dose (350 metacercariae)					
		F. gigantica		*F. hepatica*		Uninfected		*F. gigantica*		*F. hepatica*		Uninfected	
DATE	WPI	23	27	24	28	21	22	25	29	26	30	31	32
05-Apr-90	-4	23.5	24.1	33.8	23.1	23.7	24.2	33.5	41.8	35.8	34.5	31.3	22.3
12-Apr-90	-3	24.0	25.0	34.0	25.0	21.0	19.0	38.0	42.0	35.0	31.0	32.0	28.0
19-Apr-90	-2	25.1	25.1	34.4	24.7	23.5	21.0	30.5	40.6	30.9	35.9	32.5	25.5
26-Apr-90	-1	21.0	32.0	34.0	32.0	25.0	21.0	37.0	49.0	37.0	43.0	31.0	25.0
03-May-90	0	22.0	29.0	35.0	25.0	24.3	19.5	28.0	37.0	26.6	30.0	30.0	25.0
10-May-90	+1	18.5	30.5	33.2	26.2	24.3	23.5	26.2	40.1	21.6	27.4	25.9	28.6
17-May-90	+2	27.4	34.4	35.2	27.4	27.0	31.7	31.3	37.1	30.9	38.2	32.8	29.7
24-May-90	+3	28.0	39.4	39.4	32.4	28.0	34.0	55.6	43.6	44.0	43.6	35.1	26.2
31-May-90	+4	[illegible]	41.7	43.3	34.7	29.0	38.2	64.8	44.0	48.6	45.2	29.0	26.6
07-Jun-90	+5	52.1	45.2	61.4	38.2	30.1	28.9	78.7	47.5	63.7	60.2	31.2	34.7
14-Jun-90	+6	37.1	35.9	39.4	30.1	30.1	24.3	56.4	40.5	46.3	51.0	30.1	27.0
21-Jun-90	+7	42.9	38.2	55.6	36.0	30.1	34.7	52.1	40.5	44.0	52.1	35.0	29.0
28-Jun-90	+8	[illegible]	34.7	90.3	88.6	32.6	37.1	50.0	47.5	55.6	88.0	32.4	28.4
05-Jul-90	+9	[illegible]	46.3	156.3	127.4	34.7	34.7	52.1	52.1	57.9	112.9	34.7	34.7
12-Jul-90	+10	[illegible]	40.5	185.3	130.3	31.9	31.9	52.1	52.1	182.4	133.2	35.5	35.5
19-Jul-90	+11	52.1	50.3	179.5	110.0	34.7	38.2	52.1	115.8	179.5	127.4	30.1	29.0
26-Jul-90	+12	[illegible]	63.7	139.0	75.3	28.4	38.2	40.5	272.1	121.6	81.1	37.1	37.4
02-Aug-90	+13	[illegible]	92.6	167.9	46.3	33.6	41.7	34.7	212.2	69.5	63.7	35.9	32.4
09-Aug-90	+14	**	71.4	110.0	46.3	25.5	26.6	69.5	173.7	52.2	63.7	31.3	32.4
16-Aug-90	+15	**	40.5	59.8	34.7	25.9	28.6	133.2	75.3	98.4	34.5	30.1	24.3
23-Aug-90	+16	**	44.8	60.2	32.0	29.0	42.0	**	82.2	57.5	57.9	29.6	28.8
30-Aug-90	+17	**	27.8	42.8	23.2	24.3	26.6	**	52.1	44.0	78.0	27.8	24.3
06-Sep-90	+18	**	25.9	40.5	22.0	22.4	26.6	**	44.0	29.0	76.0	27.8	27.4
13-Sep-90	+19	**	25.5	39.4	23.2	23.2	27.8	**	40.5	29.0	47.5	30.1	29.0
20-Sep-90	+20	**	24.3	49.8	25.5	23.6	27.4	**	36.7	26.6	38.2	30.1	30.1

Note : ** = Sheep dead.

Appendix Table 8.2.1
Liveweights (kg) of the sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
04-Sep-91	-5	28.5	30.5	27.0	24.5	27.6	27.5	27.0	27.3	30.3	22.8	24.2	25.8
11-Sep-91	-4	28.0	30.0	27.0	25.2	27.6	28.0	26.0	27.0	30.0	22.5	24.0	25.5
18-Sep-91	-3	28.5	30.5	27.5	25.0	27.9	27.5	27.5	27.5	30.0	23.0	24.0	25.7
25-Sep-91	-2	28.0	29.0	27.5	25.0	27.4	27.0	27.5	27.3	30.5	22.5	22.0	25.0
02-Oct-91	-1	29.0	32.0	28.0	25.0	28.5	28.0	28.0	28.0	31.0	23.0	23.0	25.7
09-Oct-91	0	30.0	32.0	29.0	26.0	29.3	28.0	28.0	28.0	32.0	24.0	23.0	26.3
16-Oct-91	+1	29.0	30.0	29.0	24.0	28.0	27.0	27.0	27.0	31.0	24.0	23.0	26.0
23-Oct-91	+2	30.0	30.0	28.0	24.0	28.0	27.5	26.5	27.0	30.0	25.0	24.5	26.5
30-Oct-91	+3	29.4	30.2	27.9	25.9	28.4	26.4	27.0	26.7	31.4	22.0	23.5	25.6
06-Nov-91	+4	27.4	30.0	27.4	24.6	27.4	26.4	26.2	26.3	29.2	22.6	24.0	25.3
13-Nov-91	+5	27.4	30.0	27.6	23.9	27.2	26.1	26.5	26.3	29.6	22.5	24.9	25.7
20-Nov-91	+6	28.6	31.4	28.9	25.5	28.6	27.0	27.8	27.4	29.5	23.2	26.1	26.3
27-Nov-91	+7	26.1	31.0	30.4	26.1	28.4	27.4	27.5	27.5	29.5	23.5	26.2	26.4
04-Dec-91	+8	27.0	32.4	30.8	26.4	29.2	27.4	27.5	27.5	30.0	24.0	26.5	26.8
11-Dec-91	+9	26.9	32.2	30.1	26.5	28.9	28.2	28.9	28.6	31.1	23.5	27.0	27.2
18-Dec-91	+10	28.5	33.5	30.5	27.0	29.9	28.4	29.5	29.0	31.6	23.7	27.2	27.5
25-Dec-91	+11	28.4	33.5	31.1	26.9	30.0	29.5	29.9	29.7	31.4	23.1	27.6	27.4
01-Jan-92	+12	29.8	34.2	31.9	27.4	30.8	30.9	30.4	30.7	32.5	24.6	28.5	28.5
08-Jan-92	+13	30.0	35.3	32.2	29.0	31.6	31.9	31.3	31.6	32.2	25.2	28.0	28.5
15-Jan-92	+14	29.1	34.3	31.6	27.7	30.7	31.0	29.9	30.5	32.1	24.5	29.1	28.6
22-Jan-92	+15	28.5	34.0	33.3	28.0	31.0	31.0	29.2	30.1	31.5	25.5	28.1	28.4
29-Jan-92	+16	30.5	35.0	33.4	29.4	32.1	32.8	31.2	32.0	33.2	26.0	29.2	29.5
05-Feb-92	+17	30.2	36.2	33.6	29.6	32.4	32.4	31.4	31.9	32.5	26.8	28.0	29.1
12-Feb-92	+18	30.5	36.2	34.2	29.0	32.5	32.1	31.8	32.0	33.2	27.0	28.1	29.4
19-Feb-92	+19	30.9	36.4	34.2	28.8	32.6	32.4	31.6	32.0	34.3	27.8	28.6	30.2
26-Feb-92	+20	31.9	36.5	35.3	30.1	33.5	33.6	32.4	33.0	34.9	28.7	29.0	30.9
04-Mar-92	+21	31.0	36.0	34.6	29.3	32.7	32.5	32.0	32.3	33.0	27.9	29.0	30.0
11-Mar-92	+22	29.0	34.0	33.5	27.0	30.9	31.6	29.0	30.3	33.5	26.5	27.2	29.1
18-Mar-92	+23	29.4	33.9	32.4	28.5	31.1	31.0	28.9	30.0	33.6	26.5	27.6	29.2
25-Mar-92	+24	30.5	34.2	33.2	28.9	31.7	31.0	30.0	30.5	34.2	27.0	27.6	29.6
01-Apr-92	+25	30.6	34.2	33.4	28.9	31.8	32.6	29.5	31.1	34.4	27.0	28.0	29.8
08-Apr-92	+26	31.5	34.9	34.1	29.9	32.6	32.0	30.7	31.4	34.5	28.2	28.9	30.5
15-Apr-92	+27	30.8	35.5	34.6	29.0	32.5	31.2	29.9	30.6	34.5	28.2	28.9	30.5
22-Apr-92	+28	31.1	35.4	34.0	29.9	32.6	32.5	30.4	31.5	35.5	28.5	28.3	30.8
29-Apr-92	+29	31.4	35.4	33.0	29.9	32.4	32.5	30.5	31.5	35.3	28.1	28.5	30.6
06-May-92	+30	30.5	35.0	34.5	30.5	32.6	31.0	30.5	30.8	35.2	28.5	29.0	30.9
13-May-92	+31	31.2	35.0	34.8	29.9	32.7	31.9	30.0	31.0	35.7	28.2	28.8	30.9
20-May-92	+32	30.6	35.4	33.9	30.0	32.5	31.5	30.5	31.0	35.5	29.9	28.8	31.4
27-May-92	+33	30.3	34.5	34.0	29.3	32.0	31.2	30.4	30.8	35.6	29.7	29.8	31.7
03-Jun-92	+34	31.3	35.4	35.0	28.9	32.7	31.6	30.0	30.8	35.9	30.0	29.4	31.8
10-Jun-92	+35	31.5	34.9	33.5	28.5	32.1	32.4	29.6	31.0	36.5	29.9	29.0	31.8

Appendix Table 8.2.2
Regression statistics of the liveweights of sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

Group	Sheep	n	Line of best fit (Y=a+bX)	r2	P	Liveweight gain (kg/week)
Infected (with fluke)	11	36	Y = 28.05 + 0.10 X	0.474	< 0.0001	0.097
	14	36	Y = 31.24 + 0.15 X	0.585	< 0.0001	0.148
	15	36	Y = 28.77 + 0.20 X	0.747	< 0.0001	0.196
	16	36	Y = 25.20 + 0.15 X	0.707	< 0.0001	0.154
	Mean	36	Y = 28.34 + 0.15 X	0.722	< 0.0001	0.148*
Infected (no fluke)	12	36	Y = 27.43 + 0.17 X	0.620	< 0.0001	0.169
	13	36	Y = 27.79 + 0.10 X	0.396	< 0.0001	0.100
	Mean	36	Y = 27.63 + 0.13 X	0.546	< 0.0001	0.135*
Uninfected (controls)	17	36	Y = 30.86 + 0.03 X	0.860	< 0.0001	0.188
	18	36	Y = 22.46 + 0.21 X	0.857	< 0.0001	0.211
	19	36	Y = 24.89 + 0.15 X	0.673	< 0.0001	0.147
	Mean	36	Y = 25.67 + 0.18 X	0.920	< 0.0001	0.182*

* Differences in mean liveweight gain of the three groups were not significant ($F = 2.28$, d.f. = 2, $P = 0.108$).

Appendix Table 8.2.3
Some carcass data of the sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

	Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
Carcass weight (kg)	31.5	34.9	33.5	28.5	32.10	32.4	29.6	31.00	36.5	29.9	29.0	31.80
Dressed carcass (kg)	15.0	16.0	16.0	13.0	15.00	15.0	13.5	14.25	18.0	15.5	14.8	16.10
Dressing %	47.6	45.8	47.8	45.6	46.71	46.3	45.6	45.95	49.3	51.8	51.0	50.73
Abdominal fat (kg)	0.500	0.600	1.000	0.600	0.675	0.400	0.400	0.400	0.850	0.500	1.050	0.800
Liver weight (kg)	0.400	0.350	0.350	0.380	0.370	0.350	0.300	0.325	0.350	0.300	0.300	0.317
Liver wt. in proportion to carcass wt. (g/kg)	12.7	10.0	10.4	13.3	11.63	10.8	10.1	10.47	9.6	10.0	10.3	9.99

Appendix Table 8.2.4: *F. gigantica* faecal egg counts (EPG) of the sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
03-Sep-91	-5	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
10-Sep-91	-4	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
17-Sep-91	-3	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
24-Sep-91	-2	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
01-Oct-91	-1	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
08-Oct-91	0	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
15-Oct-91	+1	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
22-Oct-91	+2	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
29-Oct-91	+3	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
05-Nov-91	+4	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
12-Nov-91	+5	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
19-Nov-91	+6	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
26-Nov-91	+7	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
03-Dec-91	+8	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
10-Dec-91	+9	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
17-Dec-91	+10	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
24-Dec-91	+11	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
31-Dec-91	+12	0	0	0	0	0.0	0	0	0.0	0	0	0	0.0
07-Jan-92	+13	1	0	0	5	1.5	0	0	0.0	0	0	0	0.0
14-Jan-92	+14	1	0	0	6	1.8	0	0	0.0	0	0	0	0.0
21-Jan-92	+15	1	2	2	5	2.5	0	0	0.0	0	0	0	0.0
28-Jan-92	+16	5	0	0	6	2.8	0	0	0.0	0	0	0	0.0
04-Feb-92	+17	2	5	0	2	2.3	0	0	0.0	0	0	0	0.0
11-Feb-92	+18	3	4	1	1	2.3	0	0	0.0	0	0	0	0.0
18-Feb-92	+19	10	18	0	10	9.5	0	0	0.0	0	0	0	0.0
25-Feb-92	+20	50	10	5	25	22.5	0	0	0.0	0	0	0	0.0
03-Mar-92	+21	4	3	3	4	3.3	0	0	0.0	0	0	0	0.0
10-Mar-92	+22	9	7	1	4	5.3	0	0	0.0	0	0	0	0.0
17-Mar-92	+23	9	4	1	39	13.3	1	0	0.5	0	0	0	0.0
24-Mar-92	+24	7	1	1	0	2.3	0	0	0.0	0	0	0	0.0
31-Mar-92	+25	5	10	0	13	7.2	0	0	0.0	0	0	0	0.0
07-Apr-92	+26	4	0	0	5	2.3	0	0	0.0	0	0	0	0.0
14-Apr-92	+27	1	5	1	1	2.3	0	0	0.0	0	0	0	0.0
21-Apr-92	+28	0	7	5	33	11.1	1	0	0.7	0	0	0	0.0
28-Apr-92	+29	0	7	1	20	6.8	0	0	0.0	0	0	0	0.0
05-May-92	+30	0	4	0	0	1.0	0	0	0.0	0	0	0	0.0
12-May-92	+31	0	0	1	1	0.7	0	0	0.0	0	0	0	0.0
19-May-92	+32	4	3	0	0	1.6	0	0	0.0	0	0	0	0.0
26-May-92	+33	3	4	1	5	3.2	0	0	0.0	0	0	0	0.0
02-Jun-92	+34	0	7	3	13	5.7	0	0	0.0	0	0	0	0.0
09-Jun-92	+35	9	0	1	5	3.8	0	0	0.0	0	0	0	0.0

Appendix Table 8.2.5: RBC counts (x1012 cells/l) of the sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
01-Sep-91	-5	9.8	10.0	9.4	9.3	9.6	8.3	8.8	8.6	8.5	9.0	7.0	8.2
08-Sep-91	-4	9.6	10.5	9.3	9.2	9.6	8.3	8.6	8.4	8.7	8.4	6.9	8.0
15-Sep-91	-3	9.8	10.0	9.0	9.2	9.5	8.0	8.6	8.3	8.2	8.5	6.9	7.9
22-Sep-91	-2	9.2	10.1	8.4	9.2	9.2	9.1	9.5	9.3	8.7	10.0	6.0	8.2
29-Sep-91	-1	8.7	11.0	9.6	9.5	9.7	8.6	10.2	9.4	8.9	9.0	7.0	8.3
06-Oct-91	0	8.6	13.0	9.9	9.3	10.2	9.7	9.9	9.8	8.2	8.1	8.3	8.2
13-Oct-91	+1	8.2	12.7	9.7	8.7	9.8	8.9	10.3	9.6	8.0	8.1	7.3	7.8
20-Oct-91	+2	7.8	10.5	9.9	8.4	9.1	9.3	10.0	9.7	8.1	8.4	7.1	7.9
27-Oct-91	+3	8.2	11.3	9.4	9.8	9.7	10.7	9.2	9.9	8.2	8.0	8.2	8.1
03-Nov-91	+4	8.6	11.2	10.8	8.2	9.7	9.6	9.1	9.4	9.0	8.5	8.8	8.7
10-Nov-91	+5	9.1	13.0	10.0	10.3	10.6	9.9	10.0	9.9	9.9	9.6	8.1	9.2
17-Nov-91	+6	8.8	11.3	10.4	9.5	10.0	10.2	8.5	9.4	8.0	8.8	8.7	8.5
24-Nov-91	+7	9.8	11.7	10.5	9.8	10.4	11.1	10.1	10.6	9.8	9.6	9.6	9.7
01-Dec-91	+8	10.8	11.8	10.4	8.7	10.4	10.5	9.1	9.8	9.1	9.5	9.5	9.4
08-Dec-91	+9	9.0	11.5	9.0	9.2	9.7	9.9	8.8	9.3	9.0	8.9	9.9	9.3
15-Dec-91	+10	9.9	14.3	11.1	9.4	11.2	9.9	10.4	10.1	9.4	9.3	10.4	9.7
22-Dec-91	+11	10.0	13.8	11.0	10.1	11.2	10.4	11.0	10.7	10.7	9.5	10.3	10.2
29-Dec-91	+12	11.0	13.2	11.4	10.1	11.4	11.7	9.2	10.5	10.8	9.6	10.5	10.3
05-Jan-92	+13	10.3	12.3	11.2	9.4	10.8	10.9	8.9	9.9	9.8	9.6	11.0	10.1
12-Jan-92	+14	10.6	13.0	11.6	8.6	10.9	10.9	9.5	10.2	9.7	9.8	10.5	10.0
19-Jan-92	+15	9.3	11.0	11.5	10.7	10.6	11.8	9.8	10.8	9.5	9.8	10.9	10.0
26-Jan-92	+16	8.3	10.4	11.3	10.9	10.2	11.5	8.7	10.1	9.5	9.8	10.3	9.9
02-Feb-92	+17	9.8	12.0	11.0	9.8	10.6	10.3	9.1	9.7	9.1	9.5	11.1	9.9
09-Feb-92	+18	9.9	11.1	10.4	10.1	10.4	10.2	10.4	10.3	9.2	8.7	10.8	9.6
16-Feb-92	+19	9.1	12.3	10.5	10.9	10.7	11.2	10.3	10.7	10.3	9.7	10.2	10.1
23-Feb-92	+20	10.0	12.6	11.3	9.7	10.9	10.6	10.3	10.4	9.0	9.6	10.1	9.5
01-Mar-92	+21	9.6	13.3	10.9	9.6	10.8	10.2	10.5	10.3	8.4	8.7	10.2	9.1
08-Mar-92	+22	9.6	12.8	11.3	9.1	10.7	11.7	10.3	11.0	8.5	8.7	10.6	9.3
15-Mar-92	+23	9.0	10.8	10.3	8.9	9.7	9.9	8.3	9.1	10.7	9.6	10.2	10.2
22-Mar-92	+24	9.0	11.5	10.9	8.1	9.9	11.4	9.1	10.3	10.8	8.8	10.8	10.1
29-Mar-92	+25	8.8	11.4	9.6	8.5	9.6	11.1	9.3	10.2	8.8	8.6	8.8	8.7
05-Apr-92	+26	8.8	10.3	9.4	7.6	9.0	11.3	8.7	10.0	8.7	9.3	9.0	9.0
12-Apr-92	+27	8.8	12.2	9.4	7.9	9.5	11.0	9.1	10.0	9.2	8.3	8.8	8.7
19-Apr-92	+28	8.3	11.7	9.2	8.3	9.3	10.7	9.0	9.8	8.4	8.9	9.5	8.9
26-Apr-92	+29	7.5	10.0	9.0	7.5	8.5	10.8	8.9	9.8	8.6	8.3	9.2	8.7
03-May-92	+30	8.3	10.8	8.4	.8.5	9.0	11.6	9.1	10.3	8.5	9.0	9.9	9.1
10-May-92	+31	9.7	10.8	10.0	8.0	9.6	10.8	9.0	9.9	9.0	9.5	10.4	9.6
17-May-92	+32	8.9	12.3	8.9	9.3	9.9	10.7	9.7	10.2	10.0	9.3	9.9	9.7
24-May-92	+33	9.3	11.4	9.9	8.0	9.6	10.1	9.9	10.0	9.4	10.4	9.7	9.8
31-May-92	+34	9.2	11.1	9.4	8.9	9.7	10.8	9.4	10.1	9.6	9.7	9.7	9.7
07-Jun-92	+35	9.6	11.8	9.8	8.6	9.9	10.8	9.6	10.2	11.0	9.2	10.9	10.4

Appendix Table 8.2.6: Packed cell volumes (l/l) of sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
01-Sep-91	-5	0.31	0.33	0.32	0.32	0.32	0.30	0.31	0.31	0.31	0.29	0.26	0.29
08-Sep-91	-4	0.30	0.34	0.33	0.33	0.33	0.31	0.30	0.31	0.32	0.30	0.27	0.30
15-Sep-91	-3	0.32	0.35	0.32	0.33	0.33	0.30	0.30	0.30	0.33	0.30	0.27	0.30
22-Sep-91	-2	0.32	0.34	0.33	0.33	0.33	0.31	0.31	0.31	0.33	0.29	0.26	0.29
29-Sep-91	-1	0.30	0.31	0.32	0.30	0.31	0.31	0.31	0.31	0.28	0.29	0.28	0.28
06-Oct-91	0	0.32	0.33	0.31	0.30	0.32	0.30	0.31	0.31	0.28	0.28	0.29	0.28
13-Oct-91	+1	0.30	0.32	0.30	0.35	0.32	0.30	0.32	0.31	0.30	0.30	0.27	0.29
20-Oct-91	+2	0.26	0.30	0.30	0.30	0.29	0.27	0.33	0.30	0.31	0.29	0.29	0.30
27-Oct-91	+3	0.26	0.30	0.27	0.28	0.28	0.26	0.28	0.27	0.29	0.31	0.29	0.30
03-Nov-91	+4	0.30	0.35	0.29	0.29	0.31	0.28	0.28	0.28	0.31	0.28	0.28	0.29
10-Nov-91	+5	0.31	0.37	0.30	0.33	0.33	0.27	0.31	0.29	0.33	0.31	0.28	0.31
17-Nov-91	+6	0.31	0.33	0.32	0.31	0.32	0.31	0.28	0.30	0.32	0.30	0.28	0.30
24-Nov-91	+7	0.29	0.38	0.29	0.27	0.31	0.28	0.30	0.29	0.31	0.32	0.29	0.31
01-Dec-91	+8	0.33	0.35	0.31	0.29	0.32	0.30	0.30	0.30	0.30	0.30	0.28	0.29
08-Dec-91	+9	0.30	0.34	0.31	0.29	0.31	0.28	0.29	0.29	0.29	0.30	0.30	0.30
15-Dec-91	+10	0.33	0.37	0.32	0.32	0.34	0.28	0.30	0.29	0.30	0.31	0.32	0.31
22-Dec-91	+11	0.37	0.37	0.36	0.33	0.36	0.35	0.35	0.35	0.31	0.36	0.30	0.32
29-Dec-91	+12	0.36	0.35	0.36	0.34	0.35	0.30	0.30	0.30	0.35	0.31	0.29	0.32
05-Jan-92	+13	0.32	0.35	0.33	0.29	0.32	0.29	0.29	0.29	0.31	0.31	0.33	0.32
12-Jan-92	+14	0.34	0.38	0.36	0.32	0.35	0.31	0.30	0.31	0.29	0.32	0.31	0.31
19-Jan-92	+15	0.33	0.32	0.33	0.33	0.33	0.29	0.29	0.29	0.29	0.30	0.31	0.30
26-Jan-92	+16	0.29	0.31	0.33	0.33	0.32	0.31	0.28	0.30	0.29	0.31	0.30	0.30
02-Feb-92	+17	0.31	0.35	0.34	0.31	0.33	0.27	0.31	0.29	0.31	0.35	0.35	0.34
09-Feb-92	+18	0.34	0.36	0.33	0.39	0.36	0.30	0.32	0.31	0.31	0.30	0.35	0.32
16-Feb-92	+19	0.34	0.36	0.32	0.32	0.34	0.31	0.32	0.32	0.32	0.33	0.30	0.32
23-Feb-92	+20	0.33	0.35	0.33	0.33	0.34	0.38	0.35	0.37	0.30	0.31	0.27	0.29
01-Mar-92	+21	0.29	0.36	0.32	0.30	0.32	0.28	0.32	0.30	0.28	0.29	0.26	0.28
08-Mar-92	+22	0.31	0.36	0.32	0.28	0.32	0.32	0.31	0.32	0.30	0.30	0.28	0.29
15-Mar-92	+23	0.31	0.32	0.31	0.30	0.31	0.29	0.32	0.31	0.32	0.28	0.29	0.30
22-Mar-92	+24	0.31	0.34	0.31	0.30	0.32	0.32	0.30	0.31	0.31	0.32	0.28	0.30
29-Mar-92	+25	0.33	0.34	0.31	0.29	0.32	0.29	0.29	0.29	0.32	0.28	0.28	0.29
05-Apr-92	+26	0.32	0.29	0.32	0.27	0.30	0.30	0.28	0.29	0.30	0.31	0.30	0.30
12-Apr-92	+27	0.27	0.34	0.30	0.29	0.30	0.27	0.27	0.27	0.32	0.30	0.30	0.31
19-Apr-92	+28	0.27	0.33	0.29	0.28	0.29	0.29	0.28	0.29	0.30	0.29	0.31	0.30
26-Apr-92	+29	0.28	0.31	0.26	0.27	0.28	0.30	0.28	0.29	0.31	0.29	0.30	0.30
03-May-92	+30	0.29	0.29	0.28	0.29	0.29	0.33	0.31	0.32	0.29	0.31	0.30	0.30
10-May-92	+31	0.34	0.31	0.32	0.26	0.31	0.30	0.29	0.30	0.28	0.30	0.31	0.30
17-May-92	+32	0.31	0.34	0.29	0.32	0.32	0.30	0.30	0.30	0.32	0.30	0.32	0.31
24-May-92	+33	0.31	0.31	0.30	0.28	0.30	0.30	0.31	0.31	0.30	0.33	0.30	0.31
31-May-92	+34	0.31	0.32	0.29	0.31	0.31	0.30	0.30	0.30	0.31	0.31	0.30	0.31
07-Jun-92	+35	0.29	0.32	0.28	0.27	0.29	0.28	0.27	0.28	0.30	0.30	0.31	0.30

Appendix Table 8.2.7: Haemoglobin concentrations (l/l) of the sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
01-Sep-91	-5	123.2	130.0	130.0	121.0	126.1	115.0	125.0	120.0	128.0	131.0	111.0	123.3
08-Sep-91	-4	135.0	129.0	125.0	112.0	125.3	125.0	128.0	126.5	118.0	125.0	105.0	116.0
15-Sep-91	-3	146.9	123.7	131.4	115.9	129.5	108.2	123.7	116.0	131.4	115.9	109.6	119.0
22-Sep-91	-2	123.7	146.9	108.2	139.1	129.5	123.7	123.7	123.7	115.9	131.4	104.1	117.1
29-Sep-91	-1	121.6	124.6	125.3	126.0	124.4	114.3	122.4	118.4	111.3	105.4	108.1	108.3
06-Oct-91	0	121.2	133.4	119.6	119.6	123.5	119.5	103.0	111.3	104.9	105.0	109.9	106.6
13-Oct-91	+1	103.3	135.1	111.2	135.1	121.2	111.2	119.2	115.2	111.2	103.3	108.7	107.7
20-Oct-91	+2	106.2	128.1	128.1	128.1	122.6	106.2	112.6	109.4	109.9	109.9	107.6	109.1
27-Oct-91	+3	97.8	124.2	106.1	115.9	111.0	103.9	118.9	111.4	115.9	109.7	108.8	111.5
03-Nov-91	+4	109.7	138.5	115.8	115.0	119.8	121.8	119.5	120.7	124.1	113.5	103.7	113.8
10-Nov-91	+5	120.9	149.1	118.7	134.6	130.8	112.6	127.0	119.8	124.7	125.5	104.3	118.2
17-Nov-91	+6	113.6	134.2	123.6	130.4	125.5	122.0	106.0	114.0	104.5	115.9	102.2	107.5
24-Nov-91	+7	117.4	155.8	107.6	115.9	124.2	115.9	121.2	118.5	113.6	123.4	126.4	121.2
01-Dec-91	+8	125.9	142.6	119.1	117.6	126.3	115.3	117.6	116.4	117.6	119.1	113.0	116.6
08-Dec-91	+9	102.8	134.5	126.0	109.0	118.1	106.6	113.6	110.1	108.2	113.6	108.2	110.0
15-Dec-91	+10	121.8	148.3	134.7	115.8	130.1	105.2	116.5	110.8	112.7	116.5	113.5	114.2
22-Dec-91	+11	125.7	151.7	146.4	138.1	140.5	129.4	137.0	133.2	132.8	141.1	130.5	134.8
29-Dec-91	+12	151.0	150.2	137.3	130.4	142.2	122.8	109.8	116.3	137.3	122.8	117.5	125.8
05-Jan-92	+13	127.4	145.0	129.8	130.6	133.2	121.8	109.0	115.4	126.6	121.8	131.4	126.6
12-Jan-92	+14	126.0	136.0	122.1	122.9	126.8	114.4	117.5	115.9	114.4	119.0	105.9	113.1
19-Jan-92	+15	100.9	130.5	134.3	132.8	124.6	118.3	122.1	120.2	118.3	117.6	113.0	116.3
26-Jan-92	+16	110.3	126.4	118.7	120.3	118.9	123.4	125.7	124.6	116.4	118.7	110.2	115.1
02-Feb-92	+17	119.0	143.4	131.9	118.2	128.1	109.1	111.3	110.2	111.3	132.7	134.2	126.1
09-Feb-92	+18	112.5	133.9	110.9	122.8	120.0	110.9	123.6	117.3	131.5	121.2	136.3	129.7
16-Feb-92	+19	109.2	150.7	120.7	122.2	125.7	116.1	122.2	119.2	121.5	119.9	111.5	117.6
23-Feb-92	+20	116.4	150.0	135.2	132.8	133.6	107.8	125.0	116.4	118.8	118.8	106.3	114.6
01-Mar-92	+21	110.2	148.0	128.0	116.4	125.7	100.2	118.7	109.5	111.8	110.2	108.7	110.2
08-Mar-92	+22	115.5	146.1	123.1	111.6	124.1	111.6	117.8	114.7	119.4	126.2	105.5	117.0
15-Mar-92	+23	117.1	124.0	120.2	110.3	117.9	114.1	101.2	107.6	128.5	115.7	109.2	117.8
22-Mar-92	+24	115.2	138.8	122.8	107.5	121.1	119.0	106.0	112.5	122.0	118.2	103.0	114.4
29-Mar-92	+25	123.0	140.7	117.6	107.6	122.2	119.2	115.3	117.2	107.6	107.6	106.1	107.1
05-Apr-92	+26	109.1	109.8	110.6	93.8	105.8	119.0	106.8	112.9	109.1	117.5	110.6	112.4
12-Apr-92	+27	111.3	134.2	116.7	103.7	116.5	116.7	102.2	109.4	119.0	109.1	109.8	112.6
19-Apr-92	+28	105.7	150.6	122.5	110.3	122.3	111.8	114.9	113.3	124.0	133.1	120.9	126.0
26-Apr-92	+29	106.0	121.2	119.6	110.6	114.4	120.3	110.6	115.5	122.7	121.2	121.9	121.9
03-May-92	+30	100.4	115.6	107.3	111.8	108.8	121.7	107.3	114.5	111.8	114.9	121.7	116.1
10-May-92	+31	119.0	128.1	124.3	108.3	119.9	117.5	106.8	112.1	119.7	129.7	135.0	128.1
17-May-92	+32	115.8	146.0	113.5	121.8	124.3	126.4	124.8	125.6	130.1	124.1	119.5	124.6
24-May-92	+33	136.9	129.4	120.3	112.0	124.7	105.2	123.3	114.2	117.3	133.9	115.8	122.3
31-May-92	+34	128.6	124.8	130.9	136.2	130.1	121.8	132.4	127.1	122.6	121.1	114.2	119.3
07-Jun-92	+35	131.7	136.9	121.1	114.2	126.0	116.5	114.2	115.4	121.1	118.8	130.1	123.3

Appendix Table 8.2.8: Total WBC counts (x10^{9} cells/l) of sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
01-Sep-91	-5	12.5	13.5	10.5	8.5	11.25	11.3	10.7	11.00	11.6	12.0	5.8	9.80
08-Sep-91	-4	13.0	14.8	9.8	9.4	11.75	10.4	11.4	10.90	10.9	12.8	5.5	9.73
15-Sep-91	-3	13.1	15.4	10.7	7.6	11.65	11.1	12.8	11.95	12.3	12.6	4.8	9.91
22-Sep-91	-2	13.5	16.2	10.9	9.4	12.49	14.3	12.6	13.43	10.5	13.1	5.4	9.65
29-Sep-91	-1	10.7	13.2	7.8	8.0	9.92	8.2	9.7	8.95	9.9	9.3	4.8	7.98
06-Oct-91	0	12.8	12.1	10.5	10.3	11.39	12.5	10.7	11.62	9.7	8.9	4.7	7.76
13-Oct-91	+1	7.2	11.3	6.5	6.8	7.95	7.0	7.9	7.44	8.9	7.1	5.2	7.05
20-Oct-91	+2	7.4	12.7	9.8	9.3	9.79	9.1	12.2	10.68	9.9	9.8	5.8	8.49
27-Oct-91	+3	8.2	18.7	9.7	11.4	11.98	10.0	11.4	10.70	10.1	11.4	7.2	9.55
03-Nov-91	+4	10.8	15.3	8.4	10.1	11.13	9.8	9.0	9.42	9.3	11.9	6.0	9.06
10-Nov-91	+5	9.8	12.4	9.1	8.9	10.03	9.8	9.5	9.67	9.2	10.2	6.2	8.52
17-Nov-91	+6	9.2	13.0	8.5	11.6	10.57	8.6	8.8	8.70	7.3	10.4	6.8	8.17
24-Nov-91	+7	9.1	11.9	7.8	8.2	9.25	10.3	9.3	9.80	8.1	10.4	5.9	8.13
01-Dec-91	+8	9.2	11.2	8.1	6.9	8.87	8.1	7.3	7.70	7.7	10.8	6.6	8.37
08-Dec-91	+9	8.2	10.9	7.9	8.5	8.87	6.8	8.0	7.40	7.2	9.9	6.4	7.82
15-Dec-91	+10	9.7	11.6	9.0	9.5	9.94	6.1	10.8	9.45	8.6	10.0	7.3	8.61
22-Dec-91	+11	9.8	12.2	9.2	14.2	11.35	11.8	12.8	12.32	10.9	12.7	6.9	10.17
29-Dec-91	+12	10.8	11.7	9.5	9.3	10.33	7.7	8.5	8.12	8.7	8.8	7.3	8.29
05-Jan-92	+13	8.7	11.5	9.0	11.2	10.12	7.4	10.4	8.90	7.7	9.9	5.7	7.76
12-Jan-92	+14	9.6	13.1	7.3	10.1	10.01	8.3	9.4	8.85	7.2	8.7	5.2	7.03
19-Jan-92	+15	8.4	15.3	8.0	9.1	10.22	9.1	12.4	10.75	8.4	11.6	6.0	8.66
26-Jan-92	+16	13.2	19.3	8.4	12.3	13.32	11.9	13.0	12.45	7.8	9.3	5.8	7.61
02-Feb-92	+17	12.6	12.3	8.1	7.5	10.13	7.8	11.2	9.50	6.5	8.7	6.9	7.38
09-Feb-92	+18	15.1	13.7	8.2	8.4	11.35	9.8	12.3	11.03	7.1	10.4	11.8	9.77
16-Feb-92	+19	10.2	12.3	9.8	9.8	10.51	8.6	13.0	10.78	8.8	10.7	6.0	8.50
23-Feb-92	+20	8.0	12.9	8.1	8.7	9.44	8.8	9.4	9.10	8.7	8.9	4.8	7.44
01-Mar-92	+21	8.5	15.0	8.7	7.7	9.98	8.9	10.7	9.80	6.4	10.2	5.6	7.39
08-Mar-92	+22	10.5	12.1	8.5	8.1	9.82	9.3	10.3	9.77	6.7	11.9	5.7	8.12
15-Mar-92	+23	12.6	15.6	9.8	10.7	12.18	11.8	12.8	12.28	7.2	7.8	6.5	7.17
22-Mar-92	+24	7.3	10.9	6.6	6.5	7.83	8.6	8.5	8.53	6.0	7.8	5.1	6.29
29-Mar-92	+25	7.1	11.6	6.4	7.2	8.09	7.3	9.0	8.18	5.7	9.8	6.9	7.46
05-Apr-92	+26	7.7	10.7	6.6	7.2	8.05	7.6	8.1	7.88	5.9	9.1	6.4	7.16
12-Apr-92	+27	7.9	12.5	6.2	7.3	8.45	7.7	8.6	8.15	7.3	8.3	6.3	7.31
19-Apr-92	+28	8.1	11.1	7.2	8.9	8.83	8.4	8.4	8.40	6.6	10.9	6.2	7.91
26-Apr-92	+29	6.1	8.9	5.6	6.4	6.74	7.5	7.7	7.60	5.1	7.5	5.7	6.08
03-May-92	+30	7.0	10.9	6.8	7.2	7.99	6.9	8.8	7.82	6.2	9.3	8.0	7.85
10-May-92	+31	6.8	11.8	6.1	7.3	8.02	8.0	9.5	8.77	6.2	8.6	7.3	7.36
17-May-92	+32	6.3	10.1	5.9	7.8	7.52	7.8	7.3	7.55	7.2	9.2	5.4	7.26
24-May-92	+33	7.8	9.9	6.5	7.4	7.91	7.3	9.0	8.15	7.1	9.5	6.1	7.59
31-May-92	+34	15.7	8.6	8.5	7.7	10.13	7.8	7.7	7.75	5.9	8.0	5.9	6.58
07-Jun-92	+35	7.8	9.3	9.9	6.1	8.28	8.0	10.0	9.03	5.8	7.2	6.9	6.63

Appendix Table 8.2.9: Eosinophil counts (x10^{9} cells/l) of the sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
01-Sep-91	-5	0.78	1.30	0.78	0.70	0.89	0.52	0.88	0.70	0.70	0.55	0.85	0.70
08-Sep-91	-4	0.85	1.35	0.87	0.73	0.95	0.62	0.94	0.78	0.64	0.58	0.92	0.71
15-Sep-91	-3	0.82	1.17	0.59	0.49	0.77	0.49	0.82	0.66	0.49	0.89	0.83	0.74
22-Sep-91	-2	0.66	0.94	0.54	0.44	0.65	0.38	0.67	0.53	0.45	0.88	0.58	0.64
29-Sep-91	-1	0.54	0.67	0.43	0.45	0.52	0.33	0.60	0.47	0.45	0.51	0.53	0.50
06-Oct-91	0	0.47	0.58	0.47	0.43	0.49	0.27	0.38	0.32	0.48	0.32	0.48	0.43
13-Oct-91	+1	0.23	0.55	0.41	0.38	0.39	0.29	0.43	0.36	0.43	0.31	0.34	0.36
20-Oct-91	+2	0.17	1.42	0.47	0.42	0.62	0.19	0.57	0.38	0.15	0.18	0.18	0.17
27-Oct-91	+3	1.17	5.99	0.80	1.34	2.33	0.30	1.89	1.10	0.22	0.18	0.25	0.22
03-Nov-91	+4	0.96	4.51	1.83	1.29	2.15	0.61	1.07	0.84	0.15	0.25	0.23	0.21
10-Nov-91	+5	0.48	2.08	0.77	0.56	0.97	0.19	1.54	0.86	0.15	0.26	0.08	0.16
17-Nov-91	+6	0.31	2.05	0.36	0.34	0.76	0.26	0.48	0.37	0.14	0.31	0.09	0.18
24-Nov-91	+7	0.23	0.40	0.53	0.23	0.35	0.07	0.79	0.43	0.23	0.18	0.15	0.19
01-Dec-91	+8	0.55	0.54	0.72	0.51	0.58	0.14	0.71	0.43	0.27	0.12	0.20	0.20
08-Dec-91	+9	1.36	0.66	0.50	0.27	0.70	0.21	0.78	0.49	0.28	0.23	0.19	0.23
15-Dec-91	+10	1.74	2.28	0.73	1.19	1.49	0.53	1.13	0.83	0.18	0.32	0.11	0.20
22-Dec-91	+11	1.72	2.38	1.17	1.48	1.69	0.48	0.92	0.70	0.18	0.34	0.10	0.21
29-Dec-91	+12	4.87	2.37	2.07	1.23	2.63	0.40	0.94	0.67	0.30	0.27	0.25	0.27
05-Jan-92	+13	3.28	0.83	2.10	0.30	1.63	0.21	1.53	0.87	0.25	0.28	0.24	0.26
12-Jan-92	+14	2.86	1.30	0.49	0.19	1.21	0.13	0.31	0.22	0.39	0.17	0.13	0.23
19-Jan-92	+15	1.19	2.38	0.37	0.08	1.00	0.51	0.68	0.59	0.32	0.32	0.10	0.25
26-Jan-92	+16	1.83	1.09	0.30	0.23	0.86	0.37	0.78	0.58	0.30	0.23	0.18	0.24
02-Feb-92	+17	0.61	0.91	0.63	0.30	0.61	0.30	0.86	0.58	0.28	0.18	0.24	0.23
09-Feb-92	+18	1.31	0.41	0.50	0.31	0.63	0.27	0.36	0.31	0.20	0.08	0.18	0.15
16-Feb-92	+19	1.28	1.79	0.53	0.30	0.98	0.63	0.49	0.56	0.26	0.31	0.13	0.23
23-Feb-92	+20	0.95	1.43	0.60	0.39	0.84	0.84	0.31	0.58	0.24	0.26	0.22	0.24
01-Mar-92	+21	0.72	1.88	0.42	0.34	0.84	0.60	0.37	0.48	0.25	0.13	0.24	0.21
08-Mar-92	+22	0.57	1.72	0.43	0.31	0.76	1.18	1.89	1.53	0.26	0.33	0.14	0.25
15-Mar-92	+23	0.53	1.53	0.37	0.30	0.68	0.40	0.62	0.51	0.33	0.28	0.24	0.28
22-Mar-92	+24	0.55	0.70	0.69	0.35	0.57	0.36	0.62	0.49	0.32	0.24	0.25	0.27
29-Mar-92	+25	0.38	0.82	0.52	0.27	0.50	0.40	0.63	0.52	0.15	0.15	0.21	0.17
05-Apr-92	+26	0.34	0.31	0.31	0.21	0.29	0.28	0.56	0.42	0.29	0.17	0.21	0.22
12-Apr-92	+27	0.35	0.41	0.13	0.24	0.28	0.18	0.30	0.24	0.26	0.13	0.13	0.17
19-Apr-92	+28	0.33	0.33	0.28	0.14	0.27	1.23	0.27	0.75	0.13	0.22	0.22	0.19
26-Apr-92	+29	0.30	0.14	0.27	0.24	0.24	0.60	0.17	0.39	0.25	0.16	0.04	0.15
03-May-92	+30	0.44	0.13	0.34	0.26	0.29	0.27	0.17	0.22	0.33	0.06	0.22	0.20
10-May-92	+31	0.15	0.11	0.06	0.10	0.10	0.32	0.28	0.30	0.21	0.08	0.06	0.11
17-May-92	+32	0.55	0.23	0.12	0.20	0.28	0.25	0.28	0.26	0.23	0.14	0.15	0.17
24-May-92	+33	0.26	0.15	0.16	0.15	0.18	0.33	0.24	0.29	0.21	0.21	0.22	0.21
31-May-92	+34	0.24	0.23	0.32	0.26	0.26	0.37	0.57	0.47	0.21	0.21	0.11	0.18
07-Jun-92	+35	0.05	0.26	0.20	0.11	0.15	0.54	0.30	0.42	0.10	0.13	0.08	0.10

Appendix Table 8.2.10: Total serum protein (g/l) of sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
01-Sep-91	-5	58.8	60.0	59.0	60.5	59.6	60.0	63.8	61.9	59.8	60.0	56.0	58.6
08-Sep-91	-4	56.4	61.5	57.5	61.6	59.3	63.7	60.8	62.3	60.6	55.4	54.3	56.8
15-Sep-91	-3	59.0	58.5	58.2	61.1	59.2	61.6	63.6	62.6	59.3	61.3	59.8	60.1
22-Sep-91	-2	52.7	55.3	60.8	59.7	57.1	58.0	60.8	59.4	57.5	57.2	57.0	57.2
29-Sep-91	-1	59.8	60.2	60.2	63.1	60.8	57.5	69.2	63.4	63.4	62.4	62.4	62.7
06-Oct-91	0	65.7	64.3	64.3	64.3	64.7	67.5	71.5	69.5	62.5	63.4	60.9	62.3
13-Oct-91	+1	67.2	58.7	66.5	67.5	65.0	67.5	67.0	67.3	64.6	61.1	64.1	63.3
20-Oct-91	+2	62.1	59.4	65.7	68.2	63.9	63.6	61.8	62.7	65.3	63.4	65.7	64.8
27-Oct-91	+3	75.2	63.7	61.2	62.9	65.8	63.4	62.0	62.7	60.6	60.1	67.0	62.6
03-Nov-91	+4	56.5	63.1	64.8	65.8	62.6	60.6	66.3	63.5	60.1	57.3	62.4	59.9
10-Nov-91	+5	52.5	64.7	61.1	67.8	61.5	66.3	73.6	70.0	57.8	59.2	63.0	60.0
17-Nov-91	+6	56.7	61.5	57.1	61.5	59.2	55.7	61.5	58.6	58.1	58.9	65.0	60.7
24-Nov-91	+7	61.3	58.1	58.9	60.4	59.7	62.0	62.5	62.3	59.6	58.6	61.4	59.9
01-Dec-91	+8	60.3	59.5	61.5	66.5	62.0	59.5	55.0	57.3	61.7	60.3	66.9	63.0
08-Dec-91	+9	67.6	65.0	63.4	68.3	66.1	63.4	68.3	65.9	64.5	64.1	69.6	66.1
15-Dec-91	+10	59.8	63.7	60.7	60.2	61.1	65.0	67.5	66.3	61.9	59.1	59.8	60.3
22-Dec-91	+11	66.7	64.9	63.1	67.6	65.6	63.7	65.1	64.4	63.1	58.2	63.7	61.7
29-Dec-91	+12	73.8	71.6	65.3	72.4	70.8	68.8	68.0	68.4	62.5	60.0	68.3	63.6
05-Jan-92	+13	61.9	60.8	66.8	69.6	64.8	61.4	70.9	66.2	63.2	58.2	59.2	60.2
12-Jan-92	+14	58.1	62.0	61.3	62.2	60.9	57.9	70.6	64.3	65.6	52.8	65.2	61.2
19-Jan-92	+15	60.7	63.2	66.6	64.7	63.8	67.0	70.5	68.8	64.7	63.0	66.0	64.6
26-Jan-92	+16	71.2	63.4	65.3	69.3	67.3	58.5	70.6	64.6	64.9	61.9	68.7	65.2
02-Feb-92	+17	63.3	63.3	64.1	63.7	63.6	60.6	73.8	67.2	67.4	57.5	75.2	66.7
09-Feb-92	+18	60.6	60.4	62.3	61.7	61.3	66.1	70.2	68.1	63.4	60.2	72.5	65.4
16-Feb-92	+19	58.0	64.3	63.9	62.3	62.1	65.1	73.5	69.3	65.3	64.7	68.2	66.0
23-Feb-92	+20	55.5	60.8	60.0	58.6	58.7	56.0	66.1	61.1	59.8	59.1	63.5	60.8
01-Mar-92	+21	57.7	60.1	62.5	58.6	59.7	62.0	65.0	63.5	60.3	61.8	62.0	61.3
08-Mar-92	+22	56.2	59.5	66.5	63.8	61.5	59.3	65.7	62.5	69.0	61.3	64.2	64.8
15-Mar-92	+23	52.9	55.1	60.5	63.6	58.0	56.0	63.4	59.7	68.6	59.2	65.1	64.3
22-Mar-92	+24	55.5	60.7	61.5	59.2	59.2	57.9	64.9	61.4	65.0	58.6	62.8	62.1
29-Mar-92	+25	52.5	52.9	56.7	54.1	54.0	53.7	58.3	56.0	53.6	50.8	58.1	54.1
05-Apr-92	+26	53.1	58.3	58.3	57.0	56.7	57.8	69.3	63.6	62.2	55.9	61.3	59.8
12-Apr-92	+27	52.4	54.7	61.8	59.9	57.2	60.4	62.9	61.6	60.4	61.6	66.1	62.7
19-Apr-92	+28	62.0	65.6	64.1	59.8	62.9	68.5	67.0	67.8	65.4	62.4	71.3	66.4
26-Apr-92	+29	47.4	51.6	52.0	48.6	49.9	57.1	56.1	56.6	50.2	51.7	55.2	52.4
03-May-92	+30	51.2	56.5	59.8	49.4	54.2	54.2	55.3	54.8	58.8	51.4	57.5	55.9
10-May-92	+31	58.5	58.0	57.1	56.2	57.4	56.8	59.1	58.0	54.9	54.2	72.6	60.6
17-May-92	+32	48.6	53.2	54.4	49.9	51.5	49.8	58.5	54.2	52.9	53.3	55.4	53.9
24-May-92	+33	51.3	52.9	54.6	53.4	53.0	54.0	60.2	57.1	55.5	51.1	59.5	55.4
31-May-92	+34	51.3	53.5	54.9	54.1	53.5	53.2	57.3	55.3	54.1	53.2	60.1	55.8
07-Jun-92	+35	50.2	52.9	53.0	53.8	52.5	54.0	58.5	56.3	54.0	54.5	61.8	56.8

Appendix Table 8.2.11: Serum albumin concentrations (g/l) of sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
01-Sep-91	-5	25.8	26.0	25.0	26.5	25.8	26.0	26.8	26.4	27.8	26.0	26.0	26.6
08-Sep-91	-4	24.4	27.0	23.6	25.4	25.1	26.7	25.3	26.0	26.3	26.0	25.8	26.0
15-Sep-91	-3	25.8	26.0	24.5	24.6	25.2	26.1	25.3	25.7	28.3	25.2	27.4	27.0
22-Sep-91	-2	29.7	27.8	29.8	29.1	29.1	29.7	28.8	29.3	27.4	29.4	25.4	27.4
29-Sep-91	-1	29.7	29.8	29.8	29.8	29.8	30.1	28.0	29.1	31.5	30.7	25.4	29.2
06-Oct-91	0	33.4	34.1	34.8	31.2	33.4	31.4	34.9	33.2	35.0	35.7	33.4	34.7
13-Oct-91	+1	29.4	31.0	31.2	32.0	30.9	29.3	30.9	30.1	33.2	31.8	29.8	31.6
20-Oct-91	+2	29.9	30.7	30.0	28.9	29.9	27.9	30.6	29.3	30.5	29.6	28.8	29.6
27-Oct-91	+3	33.3	33.5	32.3	29.4	32.1	34.5	29.7	32.1	33.4	30.9	33.8	32.7
03-Nov-91	+4	30.1	34.8	27.8	28.8	30.4	33.6	34.4	34.0	34.2	32.0	36.5	34.2
10-Nov-91	+5	30.1	30.7	29.3	28.1	29.6	31.3	30.6	31.0	28.1	31.9	32.8	30.9
17-Nov-91	+6	32.1	28.8	30.7	27.3	29.7	34.4	32.1	33.3	31.6	30.9	34.0	32.2
24-Nov-91	+7	32.5	29.0	29.5	25.2	29.1	31.9	30.7	31.3	28.2	26.9	35.0	30.0
01-Dec-91	+8	32.8	28.5	31.6	25.9	29.7	31.0	28.0	29.5	32.9	28.6	37.8	33.1
08-Dec-91	+9	30.9	34.1	34.4	35.7	33.8	30.3	28.6	29.5	37.6	26.7	27.7	30.7
15-Dec-91	+10	34.6	34.0	34.5	31.3	33.6	33.3	33.6	33.5	30.8	35.1	35.0	33.6
22-Dec-91	+11	35.2	36.1	33.0	35.3	34.9	32.1	32.3	32.2	32.8	30.4	36.7	33.3
29-Dec-91	+12	36.6	36.3	32.3	31.4	34.2	34.2	33.6	33.9	34.6	31.0	32.8	32.8
05-Jan-92	+13	31.4	34.6	34.7	27.7	32.1	32.5	37.0	34.8	31.2	31.0	34.0	32.1
12-Jan-92	+14	32.5	36.5	34.3	32.8	34.0	35.0	29.4	32.2	31.1	33.1	36.3	33.5
19-Jan-92	+15	32.6	37.9	35.7	35.1	35.3	34.7	36.5	35.6	35.2	34.4	36.0	35.2
26-Jan-92	+16	30.0	33.4	32.7	31.3	31.9	32.9	32.7	32.8	34.3	32.4	42.4	36.4
02-Feb-92	+17	30.1	32.1	33.8	28.3	31.1	33.9	32.1	33.0	32.2	32.3	36.7	33.7
09-Feb-92	+18	30.4	33.0	33.8	30.8	32.0	34.0	33.1	33.5	35.6	33.3	39.0	36.0
16-Feb-92	+19	30.6	29.6	33.0	32.7	31.5	34.3	31.0	32.7	30.8	32.0	33.9	32.2
23-Feb-92	+20	33.1	34.5	36.2	33.3	34.3	35.3	36.1	35.7	33.9	35.2	39.7	36.2
01-Mar-92	+21	30.0	31.7	35.1	31.5	32.1	35.5	31.8	33.7	34.3	34.8	39.1	36.1
08-Mar-92	+22	28.7	29.7	33.1	31.7	30.8	31.3	30.6	30.9	31.9	32.2	33.9	32.6
15-Mar-92	+23	31.6	30.1	34.1	32.0	32.0	33.7	32.1	32.9	35.4	35.1	38.0	36.2
22-Mar-92	+24	32.2	31.5	34.1	32.2	32.5	32.2	33.2	32.7	34.1	33.6	38.2	35.3
29-Mar-92	+25	34.9	28.2	34.2	31.2	32.1	34.2	33.5	33.9	33.2	35.6	38.6	35.8
05-Apr-92	+26	28.1	30.3	32.5	26.7	29.4	31.9	33.1	32.5	29.2	31.1	34.0	31.5
12-Apr-92	+27	26.3	29.1	30.5	30.8	29.2	37.4	32.7	35.0	30.7	35.0	36.2	34.0
19-Apr-92	+28	30.6	31.1	33.2	30.0	31.2	34.0	31.7	32.9	32.4	32.9	35.3	33.5
26-Apr-92	+29	31.6	33.4	33.9	32.2	32.8	33.5	34.7	34.1	29.4	29.1	35.1	31.2
03-May-92	+30	29.1	23.8	27.4	26.1	26.6	28.6	26.9	27.7	27.6	29.2	31.8	29.5
10-May-92	+31	33.7	28.6	29.0	28.2	29.9	32.6	29.2	30.9	30.7	29.3	31.6	30.5
17-May-92	+32	35.6	28.6	30.8	33.3	32.1	29.7	31.4	30.6	30.0	32.6	37.5	33.4
24-May-92	+33	34.4	34.4	32.2	31.6	33.1	32.9	33.5	33.2	34.7	33.2	38.8	35.6
31-May-92	+34	32.4	32.2	32.2	30.2	31.7	31.7	31.3	31.5	32.7	31.0	37.5	33.7
07-Jun-92	+35	30.2	32.9	31.5	30.8	31.4	32.0	32.5	32.3	34.0	34.5	35.8	34.8

Appendix Table 8.2.12: Serum globulin concentrations (g/l) of sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
01-Sep-91	-5	33.0	34.0	34.0	34.0	33.8	34.0	37.0	35.5	32.0	34.0	30.0	32.0
08-Sep-91	-4	32.0	34.5	33.9	36.2	34.2	37.0	35.5	36.3	34.3	29.4	28.5	30.7
15-Sep-91	-3	33.2	32.5	33.7	36.5	34.0	35.5	38.3	36.9	31.0	36.1	32.4	33.2
22-Sep-91	-2	23.0	27.5	31.0	30.6	28.0	28.3	32.0	30.2	30.1	27.8	31.6	29.8
29-Sep-91	-1	30.1	30.4	30.4	33.3	31.1	27.4	41.2	34.3	31.9	31.7	37.0	33.5
06-Oct-91	0	32.3	30.2	29.5	33.1	31.3	36.1	36.6	36.4	27.5	27.7	27.5	27.6
13-Oct-91	+1	37.8	27.7	35.3	35.5	34.1	38.2	36.1	37.2	31.4	29.3	34.3	31.7
20-Oct-91	+2	32.2	28.7	35.7	39.3	34.0	35.7	31.2	33.5	34.8	33.8	36.9	35.2
27-Oct-91	+3	41.9	30.2	28.9	33.5	33.6	28.9	32.3	30.6	27.2	29.2	33.2	29.9
03-Nov-91	+4	26.4	28.3	37.0	37.0	32.2	27.0	31.9	29.5	25.9	25.3	25.9	25.7
10-Nov-91	+5	22.4	34.0	31.8	39.7	32.0	35.0	43.0	39.0	29.7	27.3	30.2	29.1
17-Nov-91	+6	24.6	32.7	26.4	34.2	29.5	21.3	29.4	25.4	26.5	28.0	31.0	28.5
24-Nov-91	+7	28.8	29.1	29.4	35.2	30.6	30.1	31.8	31.0	31.4	31.7	26.4	29.8
01-Dec-91	+8	27.5	31.0	29.9	40.6	32.3	28.5	27.0	27.8	28.8	31.7	29.1	29.9
08-Dec-91	+9	36.7	30.9	29.0	32.6	32.3	33.1	39.7	36.4	26.9	37.4	41.9	35.4
15-Dec-91	+10	25.2	29.7	26.2	28.9	27.5	31.7	33.9	32.8	31.1	24.0	24.8	26.6
22-Dec-91	+11	31.5	28.8	30.1	32.3	30.7	31.6	32.8	32.2	30.3	27.8	27.0	28.4
29-Dec-91	+12	37.2	35.3	33.0	41.0	36.6	34.6	34.4	34.5	27.9	29.0	35.5	30.8
05-Jan-92	+13	30.5	26.2	32.1	41.9	32.7	28.9	33.9	31.4	32.0	27.2	25.2	28.1
12-Jan-92	+14	25.6	25.5	27.0	29.4	26.9	22.9	41.2	32.1	34.5	19.7	28.9	27.7
19-Jan-92	+15	28.1	25.3	30.9	29.6	28.5	32.3	34.0	33.2	29.5	28.6	30.0	29.4
26-Jan-92	+16	41.1	30.0	32.6	38.0	35.4	25.7	37.9	31.8	30.6	29.5	26.3	28.8
02-Feb-92	+17	33.2	31.2	30.3	35.4	32.5	26.6	41.7	34.2	35.2	25.2	38.5	33.0
09-Feb-92	+18	30.2	27.4	28.5	30.9	29.2	32.1	37.1	34.6	27.9	26.9	33.5	29.4
16-Feb-92	+19	27.4	34.7	30.9	29.6	30.7	30.7	42.5	36.6	34.4	32.7	34.4	33.8
23-Feb-92	+20	22.4	26.2	23.9	25.3	24.5	20.7	30.0	25.4	25.9	23.9	23.9	24.5
01-Mar-92	+21	27.7	28.4	27.5	27.1	27.7	26.4	33.2	29.8	26.0	27.0	22.9	25.3
08-Mar-92	+22	27.6	29.8	33.4	32.1	30.7	28.0	35.1	31.6	37.1	29.1	30.3	32.1
15-Mar-92	+23	21.3	25.0	26.4	31.6	26.1	22.3	31.3	26.8	33.2	24.1	27.1	28.1
22-Mar-92	+24	23.3	29.2	27.4	27.0	26.7	25.7	31.7	28.7	30.9	25.0	24.7	26.9
29-Mar-92	+25	17.7	24.7	22.4	22.9	21.9	19.5	24.8	22.2	20.4	15.2	19.4	18.3
05-Apr-92	+26	25.0	28.0	25.8	30.3	27.3	25.8	36.3	31.0	33.0	24.8	27.3	28.4
12-Apr-92	+27	26.1	25.6	31.3	29.1	28.0	23.0	30.2	26.6	29.7	26.7	29.9	28.7
19-Apr-92	+28	31.5	34.4	30.9	29.8	31.6	34.6	35.3	34.9	33.0	29.5	36.0	32.8
26-Apr-92	+29	15.7	18.2	18.2	16.4	17.1	23.7	21.4	22.5	20.8	22.6	20.1	21.2
03-May-92	+30	22.1	32.6	32.4	23.4	27.6	25.6	28.4	27.0	31.3	22.1	25.7	26.4
10-May-92	+31	24.8	29.4	28.1	27.9	27.6	24.2	29.9	27.1	24.2	24.9	41.0	30.0
17-May-92	+32	12.9	24.6	23.6	16.6	19.4	20.1	27.1	23.6	22.9	20.7	17.9	20.5
24-May-92	+33	16.9	18.6	22.4	21.8	19.9	21.1	26.7	23.9	20.8	17.9	20.7	19.8
31-May-92	+34	18.9	21.3	22.7	24.0	21.7	21.5	26.1	23.8	21.3	22.2	22.6	22.1
07-Jun-92	+35	20.0	20.0	21.5	23.0	21.1	22.0	26.0	24.0	20.0	20.0	26.0	22.0

Appendix Table 8.2.13: Serum albumin to serum globulin ratios of sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
01-Sep-91	-5	0.8	0.8	0.7	0.8	0.8	0.8	0.7	0.7	0.9	0.8	0.9	0.8
08-Sep-91	-4	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.9	0.9	0.9
15-Sep-91	-3	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.9	0.7	0.8	0.8
22-Sep-91	-2	1.3	1.0	1.0	1.0	1.1	1.0	0.9	1.0	0.9	1.1	0.8	0.9
29-Sep-91	-1	1.0	1.0	1.0	0.9	1.0	1.1	0.7	0.9	1.0	1.0	0.7	0.9
06-Oct-91	0	1.0	1.1	1.2	0.9	1.1	0.9	1.0	0.9	1.3	1.3	1.2	1.3
13-Oct-91	+1	0.8	1.1	0.9	0.9	0.9	0.8	0.9	0.8	1.1	1.1	0.9	1.0
20-Oct-91	+2	0.9	1.1	0.8	0.7	0.9	0.8	1.0	0.9	0.9	0.9	0.8	0.8
27-Oct-91	+3	0.8	1.1	1.1	0.9	1.0	1.2	0.9	1.1	1.2	1.1	1.0	1.1
03-Nov-91	+4	1.1	1.2	0.8	0.8	1.0	1.2	1.1	1.2	1.3	1.3	1.4	1.3
10-Nov-91	+5	1.3	0.9	0.9	0.7	1.0	0.9	0.7	0.8	0.9	1.2	1.1	1.1
17-Nov-91	+6	1.3	0.9	1.2	0.8	1.0	1.6	1.1	1.4	1.2	1.1	1.1	1.1
24-Nov-91	+7	1.1	1.0	1.0	0.7	1.0	1.1	1.0	1.0	0.9	0.8	1.3	1.0
01-Dec-91	+8	1.2	0.9	1.1	0.6	1.0	1.1	1.0	1.1	1.1	0.9	1.3	1.1
08-Dec-91	+9	0.8	1.1	1.2	1.1	1.1	0.9	0.7	0.8	1.4	0.7	0.7	0.9
15-Dec-91	+10	1.4	1.1	1.3	1.1	1.2	1.1	1.0	1.0	1.0	1.5	1.4	1.3
22-Dec-91	+11	1.1	1.3	1.1	1.1	1.1	1.0	1.0	1.0	1.1	1.1	1.4	1.2
29-Dec-91	+12	1.0	1.0	1.0	0.8	0.9	1.0	1.0	1.0	1.2	1.1	0.9	1.1
05-Jan-92	+13	1.0	1.3	1.1	0.7	1.0	1.1	1.1	1.1	1.0	1.1	1.3	1.2
12-Jan-92	+14	1.3	1.4	1.3	1.1	1.3	1.5	0.7	1.1	0.9	1.7	1.3	1.3
19-Jan-92	+15	1.2	1.5	1.2	1.2	1.2	1.1	1.1	1.1	1.2	1.2	1.2	1.2
26-Jan-92	+16	0.7	1.1	1.0	0.8	0.9	1.3	0.9	1.1	1.1	1.1	1.6	1.3
02-Feb-92	+17	0.9	1.0	1.1	0.8	1.0	1.3	0.8	1.0	0.9	1.3	1.0	1.1
09-Feb-92	+18	1.0	1.2	1.2	1.0	1.1	1.1	0.9	1.0	1.3	1.2	1.2	1.2
16-Feb-92	+19	1.1	0.9	1.1	1.1	1.0	1.1	0.7	0.9	0.9	1.0	1.0	1.0
23-Feb-92	+20	1.5	1.3	1.5	1.3	1.4	1.7	1.2	1.5	1.3	1.5	1.7	1.5
01-Mar-92	+21	1.1	1.1	1.3	1.2	1.2	1.3	1.0	1.2	1.3	1.3	1.7	1.4
08-Mar-92	+22	1.0	1.0	1.0	1.0	1.0	1.1	0.9	1.0	0.9	1.1	1.1	1.0
15-Mar-92	+23	1.5	1.2	1.3	1.0	1.2	1.5	1.0	1.3	1.1	1.5	1.4	1.3
22-Mar-92	+24	1.4	1.1	1.2	1.2	1.2	1.2	1.0	1.1	1.1	1.3	1.5	1.3
29-Mar-92	+25	2.0	1.1	1.5	1.4	1.5	1.8	1.4	1.6	1.6	2.3	2.0	2.0
05-Apr-92	+26	1.1	1.1	1.3	0.9	1.1	1.2	0.9	1.1	0.9	1.3	1.2	1.1
12-Apr-92	+27	1.0	1.1	1.0	1.1	1.0	1.6	1.1	1.4	1.0	1.3	1.2	1.2
19-Apr-92	+28	1.0	0.9	1.1	1.0	1.0	1.0	0.9	0.9	1.0	1.1	1.0	1.0
26-Apr-92	+29	2.0	1.8	1.9	2.0	1.9	1.4	1.6	1.5	1.4	1.3	1.7	1.5
03-May-92	+30	1.3	0.7	0.8	1.1	1.0	1.1	0.9	1.0	0.9	1.3	1.2	1.1
10-May-92	+31	1.4	1.0	1.0	1.0	1.1	1.3	1.0	1.2	1.3	1.2	0.8	1.1
17-May-92	+32	2.8	1.2	1.3	2.0	1.8	1.5	1.2	1.3	1.3	1.6	2.1	1.7
24-May-92	+33	2.0	1.9	1.4	1.5	1.7	1.6	1.3	1.4	1.7	1.9	1.9	1.8
31-May-92	+34	1.7	1.5	1.4	1.3	1.5	1.5	1.2	1.3	1.5	1.4	1.7	1.5
07-Jun-92	+35	1.5	1.6	1.5	1.3	1.5	1.5	1.3	1.4	1.7	1.7	1.4	1.6

Appendix Table 8.2.14: Serum GLDH activities (U/l) of sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
01-Sep-91	-5	9.8	7.0	9.2	9.3	8.8	12.0	12.5	12.3	9.0	8.3	6.9	8.1
08-Sep-91	-4	11.2	9.2	6.5	3.7	7.7	9.3	11.0	10.2	7.3	5.5	9.1	7.3
15-Sep-91	-3	7.3	6.6	2.6	2.9	4.8	11.0	12.4	11.7	2.6	4.7	6.9	4.7
22-Sep-91	-2	12.8	1.8	7.3	5.5	6.8	12.0	15.1	13.6	1.8	5.5	14.6	7.3
29-Sep-91	-1	21.9	12.9	9.2	7.4	12.9	16.3	17.5	16.9	8.3	8.3	11.0	9.2
06-Oct-91	0	17.2	12.4	6.5	9.3	11.4	7.3	12.9	10.1	20.1	5.5	9.1	11.6
13-Oct-91	+1	3.9	6.9	10.1	7.4	7.1	7.3	14.6	11.0	9.3	8.9	11.8	10.0
20-Oct-91	+2	3.7	1.8	18.9	3.7	7.0	18.3	21.9	20.1	9.1	11.0	13.4	11.2
27-Oct-91	+3	50.7	18.3	12.8	14.6	24.1	41.7	25.6	33.6	9.3	9.1	18.3	12.2
03-Nov-91	+4	51.0	73.0	43.8	96.8	66.1	85.5	78.2	81.9	6.2	12.9	13.2	10.8
10-Nov-91	+5	63.9	118.6	191.6	106.2	120.1	59.3	82.1	70.7	7.3	9.1	11.8	9.4
17-Nov-91	+6	29.9	9.1	45.6	45.6	32.6	75.0	16.4	45.7	8.3	11.3	12.7	10.8
24-Nov-91	+7	54.8	142.4	21.9	32.9	63.0	60.3	66.6	63.5	5.5	11.0	13.7	10.0
01-Dec-91	+8	24.6	20.1	40.2	23.7	27.1	11.0	18.3	14.6	12.5	16.4	9.1	12.7
08-Dec-91	+9	36.4	113.2	29.2	31.0	52.4	90.3	27.4	58.8	13.0	18.3	13.8	15.0
15-Dec-91	+10	63.9	182.5	18.3	127.8	98.1	91.8	9.1	50.5	9.1	12.7	10.6	10.8
22-Dec-91	+11	58.3	164.3	67.4	127.8	104.4	77.6	87.6	82.6	8.8	11.3	10.7	10.3
29-Dec-91	+12	39.1	36.5	282.9	36.5	98.8	45.6	91.3	68.4	13.9	15.6	9.1	12.9
05-Jan-92	+13	45.6	82.1	49.1	39.1	54.0	36.5	56.6	46.5	18.6	12.7	17.4	16.2
12-Jan-92	+14	18.6	9.1	45.6	36.5	27.5	49.0	67.5	58.3	17.4	13.8	15.3	15.5
19-Jan-92	+15	27.4	36.5	109.5	45.6	54.7	91.2	27.4	59.3	13.0	10.4	19.2	14.2
26-Jan-92	+16	27.4	9.1	100.4	39.1	44.0	54.8	73.0	63.9	9.1	16.5	17.4	14.3
02-Feb-92	+17	47.9	104.9	136.9	109.5	99.8	36.5	31.0	33.8	17.4	12.4	7.0	12.3
09-Feb-92	+18	36.5	54.8	59.1	36.5	46.7	9.1	91.3	50.2	14.6	11.0	9.1	11.6
16-Feb-92	+19	73.0	54.8	63.9	127.8	79.8	34.7	36.5	35.6	22.0	18.6	11.0	17.2
23-Feb-92	+20	39.1	100.4	95.8	27.4	65.7	54.8	42.0	48.4	15.1	19.4	16.5	17.0
01-Mar-92	+21	63.9	121.7	18.3	73.0	69.2	41.1	73.0	57.0	21.0	16.7	18.3	18.6
08-Mar-92	+22	45.6	100.4	100.4	109.5	89.0	18.3	86.7	52.5	18.3	7.3	9.1	11.6
15-Mar-92	+23	70.0	82.1	45.6	36.5	58.6	36.5	36.5	36.5	18.3	3.7	11.0	11.0
22-Mar-92	+24	27.4	9.1	36.5	36.5	27.4	41.1	59.3	50.2	19.2	9.1	19.3	15.9
29-Mar-92	+25	91.3	84.0	36.5	9.1	55.2	50.2	42.6	46.4	25.6	5.5	23.7	18.3
05-Apr-92	+26	73.0	9.1	54.8	73.0	52.5	36.5	50.2	43.3	23.9	18.3	9.1	17.1
12-Apr-92	+27	77.6	73.0	9.1	54.8	53.6	18.3	45.6	31.9	18.4	22.1	26.5	22.3
19-Apr-92	+28	39.1	57.0	73.0	91.3	65.1	36.5	45.6	41.1	29.2	20.1	9.1	19.5
26-Apr-92	+29	27.4	77.6	77.6	23.0	51.4	49.3	36.5	42.9	22.4	18.3	14.8	18.5
03-May-92	+30	62.1	82.1	18.3	109.5	68.0	27.4	41.1	34.2	3.7	3.7	16.4	7.9
10-May-92	+31	28.8	21.9	58.4	11.0	30.0	18.3	16.4	17.3	27.4	18.3	23.8	23.2
17-May-92	+32	25.6	80.3	3.7	47.5	39.2	23.7	19.2	21.4	27.3	7.3	7.3	14.0
24-May-92	+33	29.2	23.7	21.9	71.2	36.5	24.3	21.9	23.1	20.1	14.6	27.4	20.7
31-May-92	+34	25.6	16.4	23.7	16.4	20.5	14.6	22.8	18.7	18.3	12.8	14.6	15.2
07-Jun-92	+35	38.3	11.0	38.3	18.1	26.4	14.6	11.0	12.8	13.7	19.2	17.4	16.8

Appendix Table 8.2.15: Serum GGT activities (U/l) of sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)					Infected sheep (no fluke)			Uninfected sheep (controls)			
Date	WPI	11	14	15	16	Mean	12	13	Mean	17	18	19	Mean
01-Sep-91	-5	17.4	18.3	22.3	15.4	18.4	22.8	18.6	20.7	22.3	15.8	15.7	17.9
08-Sep-91	-4	22.8	14.5	18.4	18.3	18.5	18.5	18.0	18.3	20.4	19.7	18.4	19.5
15-Sep-91	-3	18.5	10.4	24.3	13.9	16.8	15.1	19.7	17.4	17.4	25.5	11.6	18.1
22-Sep-91	-2	18.5	9.3	10.5	16.4	13.7	22.4	22.4	22.4	20.8	27.8	20.8	23.2
29-Sep-91	-1	17.4	27.8	11.6	13.5	17.6	27.8	14.0	20.9	12.3	9.3	15.1	12.2
06-Oct-91	0	29.0	14.6	29.0	25.5	24.5	10.4	18.1	14.3	19.3	27.8	13.5	20.2
13-Oct-91	+1	34.7	29.0	34.7	23.2	30.4	29.0	29.0	29.0	29.0	21.6	24.7	25.1
20-Oct-91	+2	37.1	30.5	32.4	24.3	31.1	34.7	30.1	32.4	24.3	23.3	28.2	25.3
27-Oct-91	+3	37.9	30.5	33.0	21.6	30.8	33.6	17.4	25.5	34.7	26.3	27.9	29.6
03-Nov-91	+4	31.3	31.7	26.6	27.8	29.3	32.4	30.1	31.3	26.6	24.3	28.6	26.5
10-Nov-91	+5	35.2	31.0	34.7	31.3	33.0	35.9	31.3	33.6	31.3	25.9	29.4	28.9
17-Nov-91	+6	37.1	38.2	24.3	32.4	33.0	32.4	27.8	30.1	32.4	29.0	22.9	28.1
24-Nov-91	+7	30.8	31.3	24.3	28.0	28.6	29.7	23.2	26.4	29.0	23.2	29.0	27.0
01-Dec-91	+8	34.7	40.5	29.0	34.7	34.7	30.1	25.5	27.8	24.3	26.6	26.6	25.9
08-Dec-91	+9	39.4	45.2	32.4	34.7	37.9	29.0	26.6	27.8	31.3	28.2	26.6	28.7
15-Dec-91	+10	37.1	33.6	42.8	34.7	37.1	31.0	30.1	30.6	35.9	27.8	25.5	29.7
22-Dec-91	+11	46.3	46.3	40.5	23.2	39.1	34.7	34.7	34.7	30.8	25.1	26.4	27.4
29-Dec-91	+12	98.4	75.3	69.5	133.2	94.1	57.9	63.7	60.8	30.3	29.1	28.2	29.2
05-Jan-92	+13	86.9	86.9	34.8	81.1	72.4	40.4	40.4	40.4	34.7	27.4	27.5	29.9
12-Jan-92	+14	81.1	63.7	52.1	57.9	63.7	28.9	40.4	34.7	29.6	27.4	28.9	28.6
19-Jan-92	+15	75.3	69.5	46.3	34.7	56.5	34.7	46.3	40.5	34.7	29.0	29.0	30.9
26-Jan-92	+16	69.5	75.3	40.5	46.3	57.9	34.7	23.2	29.0	29.0	28.7	29.0	28.9
02-Feb-92	+17	104.2	75.3	63.7	29.0	68.0	31.6	37.4	34.5	28.2	11.6	24.3	21.4
09-Feb-92	+18	92.6	104.2	40.5	34.7	68.0	40.5	40.5	40.5	20.4	27.4	25.7	24.5
16-Feb-92	+19	104.2	81.1	46.3	52.1	70.9	46.3	57.9	52.1	18.1	26.6	15.8	20.2
23-Feb-92	+20	139.0	81.1	40.5	52.1	78.2	115.8	57.9	86.9	16.2	13.9	15.8	15.3
01-Mar-92	+21	11.6	92.6	34.7	34.7	43.4	29.0	29.0	29.0	11.8	18.5	10.4	13.6
08-Mar-92	+22	17.4	46.3	29.0	29.0	30.4	11.6	23.2	17.4	11.6	22.1	14.2	16.0
15-Mar-92	+23	34.7	40.5	121.6	98.4	73.8	57.9	23.2	40.5	5.8	23.2	19.0	16.0
22-Mar-92	+24	40.5	34.7	23.2	34.7	33.3	23.2	75.3	49.2	12.3	16.2	8.1	12.2
29-Mar-92	+25	144.8	214.2	23.2	162.1	136.1	86.9	15.8	51.3	17.4	11.6	10.4	13.1
05-Apr-92	+26	272.1	92.6	17.4	23.2	101.3	57.9	35.8	46.8	23.2	14.0	19.7	18.9
12-Apr-92	+27	17.4	17.4	34.7	34.7	26.1	15.8	75.3	45.5	22.0	15.3	25.3	20.9
19-Apr-92	+28	156.3	69.5	63.7	40.5	82.5	29.0	23.2	26.1	17.4	13.7	25.1	18.7
26-Apr-92	+29	34.7	29.0	34.7	57.9	39.1	23.2	29.0	26.1	17.4	14.7	23.2	18.4
03-May-92	+30	11.6	75.3	52.1	5.8	36.2	17.4	52.1	34.7	18.0	17.4	19.6	18.3
10-May-92	+31	156.3	46.3	75.3	23.2	75.3	26.9	46.3	36.6	24.3	12.7	23.2	20.1
17-May-92	+32	17.4	110.0	81.1	17.4	56.5	81.1	10.4	45.7	12.7	23.2	23.2	19.7
24-May-92	+33	40.5	86.9	17.4	5.8	37.6	17.4	11.6	14.5	23.2	17.4	29.0	23.2
31-May-92	+34	69.5	34.7	5.8	75.3	46.3	11.6	5.8	8.7	29.0	11.6	34.7	25.1
07-Jun-92	+35	23.2	121.6	144.8	86.9	94.1	23.2	12.7	17.9	19.6	18.1	29.0	22.2

Appendix Table 8.2.16: Results of agar gel precipitation test on the sera of sheep
Experiment 2: Experimental infection of Nepalese Baruwal sheep with *F. gigantica*

		Infected sheep (with fluke)				Infected sheep (no fluke)		Uninfected sheep (controls)		
Date	WPI	11	14	15	16	12	13	17	18	19
01-Sep-91	-5	-	-	-	-	-	-	-	-	-
08-Sep-91	-4	-	-	-	-	-	-	-	-	-
15-Sep-91	-3	-	-	-	-	-	-	-	-	-
22-Sep-91	-2	-	-	-	-	-	-	-	-	-
29-Sep-91	-1	-	-	-	-	-	-	-	-	-
06-Oct-91	0	-	-	-	-	-	-	-	-	-
13-Oct-91	+1	-	-	-	-	-	-	-	-	-
20-Oct-91	+2	-	-	-	-	-	-	-	-	-
27-Oct-91	+3	-	-	-	-	-	-	-	-	-
03-Nov-91	+4	-	-	-	-	-	-	-	-	-
10-Nov-91	+5	-	-	-	-	-	-	-	-	-
17-Nov-91	+6	-	+	-	-	-	-	-	-	-
24-Nov-91	+7	-	-	-	+	-	-	-	-	-
01-Dec-91	+8	-	+	-	+	-	-	-	-	-
08-Dec-91	+9	-	+	-	+	-	-	-	-	-
15-Dec-91	+10	+	+	+	+	+	+	-	-	-
22-Dec-91	+11	-	+	+	+	+	+	-	-	-
29-Dec-91	+12	-	+	+	+	-	-	-	-	-
05-Jan-92	+13	+	+	+	+	-	+	-	-	-
12-Jan-92	+14	+	+	+	+	-	+	-	-	-
19-Jan-92	+15	+	+	+	+	-	-	-	-	-
26-Jan-92	+16	+	+	+	+	-	-	-	-	-
02-Feb-92	+17	+	+	+	+	-	-	-	-	-
09-Feb-92	+18	+	+	+	+	-	-	-	-	-
16-Feb-92	+19	+	+	+	+	-	-	-	-	-
23-Feb-92	+20	+	+	+	+	-	-	-	-	-
01-Mar-92	+21	+	+	+	+	-	-	-	-	-
08-Mar-92	+22	+	+	+	+	-	-	-	-	-
15-Mar-92	+23	-	+	-	+	-	-	-	-	-
22-Mar-92	+24	-	-	-	+	-	-	-	-	-
29-Mar-92	+25	-	+	-	-	-	-	-	-	-
05-Apr-92	+26	-	+	-	-	-	-	-	-	-
12-Apr-92	+27	-	-	-	-	-	-	-	-	-
19-Apr-92	+28	-	+	-	-	-	-	-	-	-
26-Apr-92	+29	-	-	-	-	-	-	-	-	-
03-May-92	+30	+	-	-	-	-	-	-	-	-
10-May-92	+31	-	-	-	-	-	-	-	-	-
17-May-92	+32	-	-	-	-	-	-	-	-	-
24-May-92	+33	-	+	-	-	-	-	-	-	-
31-May-92	+34	-	-	-	-	-	-	-	-	-
07-Jun-92	+35	-	-	-	-	-	-	-	-	-

+ = positive for precipitation lines; - = negative for precipitation lines.

Appendix Table 8.3.1
Liveweights (kg) of the individual goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)								Infected goats (Group B)								Un'afected goats (Group C)								Statistics	
Date	WPI	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	H	P
07-Sep-91	-5	18.2	15.0	16.5	17.3	20.2	18.0	17.5	0.7	18.5	14.8	16.3	17.0	21.0	21.0	18.1	0.9	18.6	14.8	16.5	15.0	20.3	17.4	17.1	0.8	0.4	0.8372
11-Sep-91	-4	18.5	15.5	16.5	17.5	20.5	18.5	17.8	0.7	18.5	14.5	16.5	17.5	21.0	21.5	18.3	1.0	18.5	14.5	16.5	15.5	20.5	17.5	17.2	0.8	-	-
18-Sep-91	-3	19.5	15.5	17.2	16.0	20.5	16.0	17.5	0.8	19.5	15.0	17.0	16.5	21.5	21.0	18.4	1.0	19.0	14.2	16.5	15.0	21.5	17.5	17.3	1.0	-	-
25-Sep-91	-2	20.5	15.5	17.5	16.5	20.5	16.5	17.8	0.8	20.0	15.5	16.5	17.0	20.5	20.0	18.3	0.8	19.3	14.5	16.4	15.2	21.3	16.8	17.3	1.0	-	-
02-Oct-91	-1	20.5	14.5	17.5	17.5	21.5	16.5	18.0	1.0	20.5	15.5	16.5	17.5	20.5	20.0	18.4	0.8	19.5	15.0	16.5	15.5	21.5	17.5	17.6	0.9	-	-
09-Oct-91	0	20.5	15.5	17.5	17.5	20.5	17.5	18.2	0.7	20.5	15.5	16.5	17.5	20.5	19.5	18.3	0.8	19.5	15.2	16.5	15.5	21.0	17.5	17.5	0.9	0.6	0.7471
16-Oct-91	+1	20.5	15.5	16.5	16.5	20.5	16.5	17.7	0.8	20.5	15.5	16.5	16.5	21.0	20.0	18.3	0.9	19.5	14.5	15.5	15.5	21.5	17.5	17.3	1.0	-	-
23-Oct-91	+2	21.0	14.5	18.0	17.0	20.5	17.0	18.0	0.9	20.0	16.0	17.0	16.5	20.0	20.5	18.3	0.8	18.5	14.5	16.0	16.5	21.5	17.0	17.3	0.9	-	-
30-Oct-91	+3	21.0	14.9	18.1	17.0	21.0	16.5	18.1	0.9	19.9	15.9	16.4	16.8	20.2	20.5	18.3	0.8	19.5	14.4	16.0	16.5	21.0	17.0	17.4	0.9	-	-
06-Nov-91	+4	21.5	14.7	19.0	17.2	21.1	16.8	18.4	1.0	20.6	16.0	17.4	16.0	21.3	20.2	18.6	0.9	19.6	15.6	15.6	16.6	22.5	16.8	17.8	1.0	-	-
13-Nov-91	+5	22.5	14.8	19.0	17.3	20.9	17.5	18.7	1.0	20.8	16.8	17.6	16.9	21.5	20.9	19.1	0.8	19.8	15.9	16.3	17.9	22.8	16.7	18.2	1.0	0.6	0.7279
20-Nov-91	+6	22.2	14.6	19.2	17.6	21.5	17.6	18.8	1.0	21.4	16.6	17.5	17.0	22.1	20.4	19.2	0.9	20.4	16.3	16.0	17.4	23.3	17.1	18.4	1.1	-	-
27-Nov-91	+7	22.1	14.4	19.5	17.0	20.8	18.3	18.7	1.0	21.5	16.9	18.3	17.3	22.0	20.8	19.5	0.8	20.5	16.4	16.5	18.1	23.3	17.9	18.8	1.0	-	-
04-Dec-91	+8	23.3	14.8	20.4	18.1	22.5	19.0	19.7	1.2	23.8	17.8	18.8	18.2	23.2	21.6	20.6	1.0	21.8	16.8	17.4	19.2	24.5	18.5	19.7	1.1	-	-
11-Dec-91	+9	23.7	14.7	20.2	18.1	23.0	19.8	19.9	1.2	23.8	17.8	19.4	19.0	24.1	22.4	21.1	1.0	21.8	17.5	18.2	19.2	25.5	19.5	20.3	1.1	-	-
18-Dec-91	+10	24.5	15.9	22.0	18.9	22.6	19.3	20.5	1.2	24.4	17.5	19.9	19.6	24.5	22.4	21.4	1.1	22.4	18.0	18.6	20.0	25.7	19.6	20.7	1.1	0.3	0.8725
25-Dec-91	+11	24.2	15.7	21.5	18.0	21.6	19.5	20.1	1.1	24.2	18.0	19.3	20.2	24.0	23.0	21.5	1.0	22.4	18.7	19.2	20.4	25.5	20.5	21.1	0.9	-	-
01-Jan-92	+12	25.2	15.8	21.2	19.0	21.9	21.5	20.8	1.2	25.5	19.0	20.2	21.9	25.2	23.5	22.6	1.0	23.5	19.9	20.5	21.0	26.1	21.4	22.1	0.9	-	-
08-Jan-92	+13	25.5	15.6	21.7	19.0	21.7	21.1	20.8	1.2	25.7	19.0	20.3	22.5	26.0	24.5	23.0	1.1	23.7	20.5	21.5	21.4	26.8	22.2	22.7	0.9	-	-
15-Jan-92	+14	26.3	15.5	21.4	18.9	21.2	20.0	20.6	1.3	26.5	18.6	20.4	22.0	26.2	24.2	23.0	1.2	24.5	20.4	21.2	21.6	27.5	22.6	23.0	1.0	-	-
22-Jan-92	+15	26.1	15.3	22.0	19.0	21.0	20.3	20.6	1.3	26.4	18.9	21.1	22.0	26.7	24.0	23.2	1.1	24.4	21.5	21.2	22.0	27.8	22.2	23.2	0.9	2.6	0.2746
29-Jan-92	+16	27.4	15.5	23.0	19.0	21.1	21.4	21.2	1.5	27.5	20.0	22.3	23.2	27.5	25.4	24.3	1.1	25.5	22.0	22.2	22.6	28.6	22.8	24.0	1.0	-	-
05-Feb-92	+17	27.5	16.6	22.6	19.0	20.6	20.8	21.2	1.4	28.0	20.6	22.1	23.6	27.0	25.4	24.5	1.1	26.0	22.5	22.8	23.0	28.5	23.3	24.4	0.9	-	-
12-Feb-92	+18	29.0	16.5	23.0	19.0	20.6	22.1	21.7	1.6	28.5	20.4	23.0	23.8	28.0	26.4	25.0	1.2	26.5	22.5	22.8	23.5	29.0	23.8	24.7	0.9	-	-
19-Feb-92	+19	28.1	16.4	23.5	18.4	20.6	21.8	21.5	1.5	28.9	20.7	23.1	23.6	28.2	26.0	25.1	1.2	26.9	23.0	23.2	23.8	29.1	24.0	25.0	0.9	-	-
26-Feb-92	+20	28.6	16.6	23.4	18.9	20.7	22.5	21.8	1.5	30.0	20.6	23.3	24.8	29.1	27.4	25.9	1.3	27.0	23.2	23.5	24.3	29.9	24.3	25.4	1.0	4.3	0.1167
04-Mar-92	+21	29.0	17.6	23.9	18.3	20.6	23.0	22.1	1.6	29.3	21.3	23.6	25.6	29.2	28.0	26.2	1.2	27.3	23.6	24.5	25.0	30.4	24.8	25.9	0.9	-	-
11-Mar-92	+22	29.7	17.0	23.2	18.0	19.5	22.5	21.7	1.7	30.3	20.6	23.2	24.6	28.9	26.6	25.7	1.4	27.3	23.5	24.2	24.8	29.9	25.0	25.8	0.9	-	-
18-Mar-92	+23	29.2	17.2	24.1	18.4	19.6	22.0	21.8	1.6	30.2	20.8	23.9	25.5	30.2	26.8	26.2	1.4	28.2	24.5	24.9	25.4	31.1	25.2	26.6	1.0	-	-
25-Mar-92	+24	30.5	17.5	24.9	18.9	19.6	23.8	22.5	1.8	31.2	21.4	24.5	26.2	31.4	27.5	27.0	1.4	29.2	24.8	25.4	25.7	32.1	26.5	27.3	1.1	-	-
01-Apr-92	+25	29.2	16.5	24.2	19.3	18.8	23.8	22.0	1.7	31.2	21.0	24.3	26.4	31.2	26.1	26.7	1.5	29.0	25.0	25.6	26.2	32.4	26.5	27.5	1.0	5.6	0.0599
08-Apr-92	+26	30.4	17.4	25.0	19.2	18.8	24.0	22.5	1.8	31.5	21.6	24.8	27.2	32.0	27.9	27.5	1.5	29.5	25.1	26.0	26.8	32.8	27.1	27.9	1.1	-	-
15-Apr-92	+27	30.9	16.4	25.7	20.0	18.3	24.9	22.7	2.0	32.3	22.3	25.0	27.3	31.5	28.5	27.8	1.4	30.2	25.5	26.9	28.5	33.0	27.4	28.6	1.0	-	-
22-Apr-92	+28	32.4	16.6	26.8	20.0	18.4	25.0	23.2	2.2	33.2	22.8	26.0	28.4	32.5	29.4	28.7	1.5	31.5	26.0	27.1	29.4	33.5	28.5	29.3	1.0	-	-
29-Apr-92	+29	30.5	16.4	27.4	19.5	17.9	25.5	22.9	2.1	33.5	22.6	25.5	28.5	32.1	29.5	28.6	1.5	31.6	26.2	27.1	29.0	33.6	28.0	29.3	1.1	-	-
06-May-92	+30	31.5	16.4	26.2	19.5	17.6	25.3	22.8	2.2	33.6	22.5	25.5	29.3	33.2	28.9	28.8	1.6	32.0	26.5	27.5	29.0	34.0	28.5	29.6	1.1	5.6	0.0622
13-May-92	+31	33.3	16.4	27.5	20.9	17.2	25.5	23.5	2.4	35.2	23.5	26.0	29.6	33.4	29.4	29.5	1.6	32.8	26.9	28.0	29.8	34.6	29.4	30.3	1.1	-	-
20-May-92	+32	31.5	16.0	27.2	20.8	17.2	24.4	22.9	2.2	34.8	23.2	26.0	29.6	34.0	29.8	29.6	1.7	32.4	27.4	28.3	30.5	34.6	29.8	30.5	1.0	-	-
27-May-92	+33	32.2	15.5	28.2	21.0	17.6	25.0	23.3	2.4	35.5	23.5	26.0	29.8	34.5	30.0	29.9	1.7	32.8	27.9	28.2	30.4	35.0	30.5	30.8	1.0	-	-
03-Jun-92	+34	32.4	**	27.8	21.3	17.3	25.2	24.8	2.6	35.4	23.8	26.4	29.4	34.0	30.5	29.9	1.6	33.0	28.0	28.4	30.8	35.0	30.4	30.9	1.0	-	-
10-Jun-92	+35	32.8	**	27.0	21.0	17.0	25.4	24.6	2.7	35.9	24.0	26.8	29.5	34.8	31.0	30.3	1.7	33.2	28.3	28.8	31.2	35.2	30.7	31.2	1.0	4.2	0.1240

Statistics = results of Kruskal Wallis test; H = H statistic; P = significance (probability); - = analysis not done; ** = animal dead, the weeks when the liveweights of the groups were significantly different are printed in bold faces.

Appendix Table 8.3.2
Regression statistics of the liveweight of individual animals
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

Group	Goat	n	Line of best fit (Y=a+bX)	r2	P	Liveweight gain (kg/week)
A	31	36	Y = 20.465+0.380X	0.970	< 0.0001	0.380
	32	34	Y = 14.833+0.065X	0.499	< 0.0001	0.065
	33	36	Y = 17.494+0.305X	0.969	< 0.0001	0.305
	34	36	Y = 16.929+0.106X	0.805	< 0.0001	0.106
	35	36	Y = 22.369+0.133X	0.690	< 0.0001	0.133
	36	36	Y = 16.599+0.277X	0.951	< 0.0001	0.277
	Mean	36	Y = 18.005+0.178X	0.957	< 0.0001	0.178
B	41	36	Y = 19.256+0.489X	0.989	< 0.0001	0.489
	42	36	Y = 15.452+0.249X	0.980	< 0.0001	0.249
	43	36	Y = 16.330+0.317X	0.979	< 0.0001	0.317
	44	36	Y = 15.569+0.437X	0.974	< 0.0001	0.437
	45	36	Y = 19.752+0445X	0.989	< 0.0001	0.445
	46	36	Y = 19.410+0.334X	0.970	< 0.0001	0.334
	Mean	36	Y = 17.644+0.378X	0.989	< 0.0001	0.378
	51	36	Y = 18.072+0.449X	0.989	< 0.0001	0.449
	52	36	Y = 14.224+0.423X	0.982	< 0.0001	0.423
	53	36	Y = 14.851+0.424X	0.979	< 0.0001	0.424
	54	36	Y = 15.149+0.465X	0.994	< 0.0001	0.465
	55	36	Y = 20.913+0.438X	0.990	< 0.0001	0.437
	56	36	Y = 15.774+0.433X	0.983	< 0.0001	0.433
	Mean	36	Y = 16.493+0.439X	0.993	< 0.0001	0.439

Appendix Table 8.3.3
Comparisions of the slopes (b) of regression lines of liveweights against WPI
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

Comparision	d.f.	Slopes (b)	F value	t value	Probability (P)
Groups A, B and C	2	0.18, 0.38 and 0.44	560.00	23.664	< 0001
Group A vs Group B	1	0.18 vs 0.38	77.59	8.808	< 0001
Group A vs Group C	1	0.18 vs 0.44	132.67	11.518	< 0001
Group B vs Group C	1	0.38 vs 0.44	43.36	6.603	< 0001

Appendix Table 8.3.4
Some carcass data of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

	Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	F	P
Carcass wt (kg)	33.0	15.5	27.5	21.0	17.8	25.5	23.38	2.43	36.2	24.0	27.0	29.5	33.4	31.5	30.27	1.64	33.5	28.5	29.0	31.5	35.2	30.5	31.37	0.97	4.90	0.0230
Dressed carcass (kg) ..	17.0	6.0	13.0	10.0	7.0	12.0	10.83	1.52	18.0	11.5	13.0	15.0	17.0	15.0	14.92	0.90	16.5	15.0	14.0	16.0	18.0	14.8	15.72	0.53	5.11	0.0203
Killing out %	51.5	38.7	47.3	47.6	39.3	47.1	45.25	1.90	49.7	47.9	48.1	50.8	50.9	47.6	49.19	0.56	49.3	52.6	48.3	50.8	51.1	48.5	50.10	0.63	3.71	0.0491
Abdominal fat (kg)......	1.25	0.05	1.20	0.35	0.10	1.00	0.66	0.21	2.70	1.00	0.80	1.70	1.70	1.25	1.53	0.25	0.95	1.50	1.20	1.60	1.70	1.50	1.41	0.10	4.69	0.0262
Weight of liver (kg)	0.45	0.40	0.50	0.40	0.50	0.45	0.45	0.02	0.50	0.49	0.45	0.50	0.45	0.48	0.46	0.01	0.40	0.45	0.38	0.45	0.48	0.40	0.43	0.01	1.25	0.3156
Liver wt. in proportion to carcass wt. (g/kg)...	13.6	25.8	18.2	19.0	28.1	17.6	20.40	2.03	13.8	16.7	16.7	16.9	13.5	15.2	15.47	0.57	11.9	15.8	13.1	14.3	13.6	13.1	13.65	0.49	6.46	0.0095

Statistics = results of one-way analysis of variance (ANOVA); F = F statistic; P = significance (probability).

Appendix Table 8.3.5
Fasciola gigantica faecal egg counts (EPG) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
Date	WPI	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	U	P
03-Sep-91	-5	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
10-Sep-91	-4	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
17-Sep-91	-3	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
24-Sep-91	-2	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
01-Oct-91	-1	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
08-Oct-91	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
15-Oct-91	+1	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
22-Oct-91	+2	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
29-Oct-91	+3	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
05-Nov-91	+4	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
12-Nov-91	+5	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
19-Nov-91	+6	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
26-Nov-91	+7	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
03-Dec-91	+8	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
10-Dec-91	+9	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
17-Dec-91	+10	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
24-Dec-91	+11	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
31-Dec-91	+12	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0.0	0.0	-	-
07-Jan-92	+13	4	5	4	10	1	0	4.0	1.3	1	0	3	0	1	0	0.8	0.4	0	0	0	0	0	0	0.0	0.0	7.5	0.0931
14-Jan-92	**+14**	1	2	1	3	3	0	1.7	0.5	1	0	0	0	0	1	0.4	0.2	0	0	0	0	0	0	0.0	0.0	-	-
21-Jan-92	**+15**	2	8	7	15	2	2	6.0	1.9	1	0	1	0	2	0	0.7	0.3	0	0	0	0	0	0	0.0	0.0	1.5	0.0043
28-Jan-92	**+16**	9	19	15	10	35	3	15.2	4.2	1	0	1	0	0	0	0.3	0.2	0	0	0	0	0	0	0.0	0.0	-	-
04-Feb-92	**+17**	2	17	35	2	59	5	20.0	8.5	4	1	1	2	0	3	1.8	0.5	0	0	0	0	0	0	0.0	0.0	-	-
11-Feb-92	**+18**	3	28	10	4	10	5	10.0	3.5	1	0	0	1	0	0	0.3	0.2	0	0	0	0	0	0	0.0	0.0	-	-
18-Feb-92	**+19**	3	17	11	2	16	3	8.7	2.6	3	0	0	2	0	4	1.5	0.7	0	0	0	0	0	0	0.0	0.0	-	-
25-Feb-92	**+20**	3	15	27	18	78	5	24.3	10.3	2	0	0	1	0	0	0.5	0.3	0	0	0	0	0	0	0.0	0.0	0.0	0.0022
03-Mar-92	**+21**	1	10	7	14	31	0	10.5	4.2	0	0	1	0	0	1	0.4	0.2	0	0	0	0	0	0	0.0	0.0	-	-
10-Mar-92	**+22**	1	14	10	3	4	0	5.4	2.1	4	3	3	1	0	0	1.8	0.6	0	0	0	0	0	0	0.0	0.0	-	-
17-Mar-92	**+23**	3	1	12	1	17	7	6.7	2.4	1	0	1	0	0	3	0.9	0.4	0	0	0	0	0	0	0.0	0.0	-	-
24-Mar-92	**+24**	9	3	0	10	22	1	7.6	3.1	0	1	0	0	1	3	0.9	0.4	0	0	0	0	0	0	0.0	0.0	-	-
31-Mar-92	**+25**	7	12	1	4	9	3	5.9	1.5	3	0	1	0	0	0	0.7	0.4	0	0	0	0	0	0	0.0	0.0	2.0	0.0087
07-Apr-92	**+26**	2	4	10	11	3	3	5.4	1.5	5	0	0	0	0	1	1.1	0.8	0	0	0	0	0	0	0.0	0.0	-	-
14-Apr-92	**+27**	5	10	9	3	75	3	17.5	10.6	3	0	9	1	0	0	2.2	1.3	0	0	0	0	0	0	0.0	0.0	-	-
21-Apr-92	**+28**	1	1	28	5	39	3	12.9	6.1	1	1	4	1	1	3	2.0	0.4	0	0	0	0	0	0	0.0	0.0	-	-
28-Apr-92	**+29**	5	5	7	7	21	0	7.4	2.6	0	0	1	0	0	5	1.1	0.8	0	0	0	0	0	0	0.0	0.0	-	-
05-May-92	**+30**	3	15	10	3	17	0	7.9	2.7	0	3	1	0	0	4	1.3	0.6	0	0	0	0	0	0	0.0	0.0	5.5	0.0411
12-May-92	**+31**	0	1	1	3	12	1	3.0	1.6	1	3	0	0	1	0	0.9	0.4	0	0	0	0	0	0	0.0	0.0	-	-
19-May-92	**+32**	0	3	3	1	4	0	1.7	0.6	0	5	1	1	0	0	1.3	0.8	0	0	0	0	0	0	0.0	0.0	-	-
26-May-92	**+33**	4	**	4	8	35	0	8.5	5.0	5	0	0	3	0	0	1.3	0.8	0	0	0	0	0	0	0.0	0.0	-	-
02-Jun-92	**+34**	3	**	18	7	34	9	14.0	5.6	4	0	1	1	0	0	1.1	0.6	0	0	0	0	0	0	0.0	0.0	-	-
09-Jun-92	**+35**	11	**	7	4	29	1	10.4	4.9	0	1	3	0	0	4	3.9	0.6	0	0	0	0	0	0	0.0	0.0	3.0	0.0303

Statistics = results of Mann Whitney test; U = U statistic; P = significance (probability); - = analysis not done; ** = animal dead, the weeks when the faecal egg counts in the groups A and B were significantly different are printed in bold faces.

Appendix Table 8.3.6: RBC counts (x1012 cell/l) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

Date	WPI	Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
		31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	11.8	12.3	9.5	11.8	12.2	12.3	11.65	0.40	11.5	9.8	10.5	11.4	11.0	9.5	10.62	0.31	11.2	10.6	10.5	11.3	10.8	11.2	10.93	0.13	5.38	0.0678
08-Sep-91	-4	12.4	11.8	9.8	11.7	12.5	11.9	11.68	0.36	12.2	9.5	10.4	11.2	10.8	9.8	10.65	0.37	11.0	11.0	10.7	11.5	10.8	10.9	10.98	0.10	-	-
15-Sep-91	-3	12.9	11.8	9.6	11.9	12.0	11.3	11.60	0.41	11.1	9.4	10.2	11.5	10.3	9.9	10.40	0.29	11.3	10.5	10.5	11.7	10.6	11.4	11.00	0.20	-	-
22-Sep-91	-2	12.2	12.7	9.5	11.7	11.6	12.6	11.71	0.44	12.3	9.3	10.5	11.4	11.1	9.8	10.73	0.40	10.3	10.8	10.3	11.7	11.2	11.2	10.90	0.20	-	-
29-Sep-91	-1	11.8	11.4	10.2	11.1	12.7	13.3	11.74	0.42	11.6	9.2	10.4	10.2	10.2	10.2	10.29	0.29	10.3	10.7	11.9	11.8	10.6	10.8	11.01	0.26	-	-
06-Oct-91	0	13.3	11.8	10.4	11.7	13.8	13.6	12.43	0.49	13.1	10.8	12.0	12.3	10.8	10.3	11.56	0.41	10.7	10.7	11.1	12.0	10.4	11.1	11.01	0.20	3.32	0.1903
13-Oct-91	+1	14.3	11.2	11.2	11.8	12.9	13.5	12.47	0.48	13.7	10.6	12.1	11.3	10.1	10.6	11.41	0.49	11.5	10.4	11.5	11.5	10.0	11.4	11.07	0.25	-	-
20-Oct-91	+2	15.2	12.0	10.5	11.0	14.1	14.6	12.90	0.74	14.2	12.0	12.0	12.1	12.4	12.6	12.55	0.32	11.7	10.7	12.0	12.9	15.2	12.1	12.43	0.58	-	-
27-Oct-91	+3	15.7	12.8	12.0	12.8	13.7	15.6	13.77	0.58	15.6	12.8	12.3	13.1	12.9	12.6	13.20	0.45	12.7	11.1	13.0	14.3	16.9	12.3	13.37	0.74	-	-
03-Nov-91	+4	14.9	12.2	12.2	12.6	12.8	14.9	13.27	0.48	15.2	13.0	13.4	12.7	15.9	13.8	13.99	0.48	11.0	11.5	12.8	14.5	14.8	11.4	12.68	0.61	-	-
10-Nov-91	+5	14.8	12.3	12.8	12.7	13.4	14.3	13.36	0.37	14.9	13.4	14.3	12.7	13.9	14.2	13.90	0.28	12.9	12.4	13.2	14.3	14.9	11.4	13.20	0.48	1.18	0.5557
17-Nov-91	+6	13.8	12.2	12.5	12.1	13.1	14.1	12.96	0.32	13.4	12.2	12.6	13.4	13.5	13.0	13.03	0.20	13.2	12.1	12.7	13.0	14.4	11.7	12.84	0.35	-	-
24-Nov-91	+7	14.8	12.5	13.6	13.7	13.4	17.8	14.28	0.70	15.0	12.6	13.2	13.7	13.9	12.6	13.47	0.34	14.1	12.3	12.1	12.6	14.8	13.1	13.18	0.40	-	-
01-Dec-91	+8	15.0	11.1	12.3	12.8	14.1	15.6	13.49	0.64	14.4	12.0	11.7	15.1	15.1	12.9	13.52	0.56	12.0	13.3	13.4	13.8	15.1	12.2	13.29	0.43	-	-
08-Dec-91	+9	13.7	11.4	12.2	14.1	12.1	15.7	13.18	0.60	14.5	11.9	13.0	12.0	15.0	12.1	13.09	0.50	13.0	13.5	13.2	15.4	13.9	11.5	13.43	0.47	-	-
15-Dec-91	+10	16.2	12.1	13.4	15.3	12.0	14.2	13.86	0.63	13.0	12.9	14.0	12.9	15.8	12.7	13.54	0.45	13.5	15.1	14.1	15.4	15.1	11.2	14.04	0.59	0.67	0.7160
22-Dec-91	+11	17.1	13.5	13.2	15.1	11.2	17.5	14.59	0.90	14.7	13.1	15.3	14.2	16.6	13.2	14.50	0.49	13.2	15.3	13.7	16.3	15.0	12.7	14.35	0.52	-	-
29-Dec-91	+12	15.4	14.4	11.3	13.4	8.0	15.4	12.96	1.08	13.5	12.8	15.6	13.9	15.9	13.3	14.14	0.47	13.0	15.0	13.3	16.6	15.2	13.4	14.40	0.52	-	-
05-Jan-92	+13	15.3	10.6	10.2	12.4	7.5	15.9	11.98	1.20	13.6	12.9	14.9	14.7	15.1	13.4	14.09	0.34	13.7	15.9	12.6	16.8	14.9	14.4	14.72	0.56	2.57	0.2767
12-Jan-92	+14	15.6	11.0	9.3	12.5	8.0	16.2	12.08	1.24	13.0	13.2	14.5	14.8	15.7	13.5	14.11	0.40	12.2	15.8	12.5	17.3	14.4	14.5	14.44	0.72	-	-
19-Jan-92	+15	16.5	11.9	10.0	11.0	9.3	13.4	11.99	0.97	14.6	12.8	15.3	15.3	10.9	12.3	13.52	0.68	14.4	16.4	13.4	17.4	15.6	14.9	15.34	0.52	5.25	0.0725
26-Jan-92	+16	15.4	12.3	10.6	11.7	8.9	13.8	12.09	0.85	14.0	11.9	13.2	14.1	14.7	14.0	13.65	0.37	14.3	15.6	13.5	16.7	15.2	14.3	14.94	0.43	-	-
02-Feb-92	+17	15.6	12.4	10.8	11.7	8.5	15.2	12.37	1.01	14.6	12.6	16.5	13.6	16.8	10.6	14.12	0.89	12.5	17.0	14.5	19.8	14.4	14.4	15.42	0.96	-	-
09-Feb-92	+18	15.9	12.0	10.6	10.7	7.8	14.9	11.97	1.11	15.1	12.9	14.9	14.3	15.1	12.6	14.13	0.41	13.6	16.2	13.5	17.8	14.7	14.7	15.09	0.62	-	-
16-Feb-92	+19	15.7	13.7	13.3	12.7	8.1	17.0	13.41	1.15	15.3	14.8	16.4	14.0	16.4	15.0	15.32	0.36	15.7	16.7	11.6	16.5	16.3	16.8	15.60	0.75	-	-
24-Feb-92	+20	17.7	13.6	12.0	12.4	7.9	16.0	13.23	1.27	15.1	13.7	13.9	14.6	14.2	12.1	13.92	0.38	13.6	15.8	13.4	17.0	14.4	14.7	14.79	0.51	1.18	0.5544
01-Mar-92	+21	14.9	13.6	10.6	12.6	6.4	15.0	12.16	1.22	14.5	12.6	14.5	13.4	12.6	12.9	13.42	0.33	13.1	15.2	13.0	16.6	15.3	14.8	14.66	0.52	-	-
08-Mar-92	+22	14.6	12.9	11.3	11.8	7.3	13.7	11.94	0.96	14.3	12.8	14.2	13.1	13.8	13.2	13.57	0.23	13.4	13.3	14.1	16.6	14.6	15.1	14.51	0.46	-	-
15-Mar-92	+23	14.2	13.2	11.1	12.1	7.3	15.4	12.21	1.05	13.4	11.7	14.0	14.3	14.2	11.5	13.19	0.47	12.7	14.4	13.1	16.5	15.6	14.1	14.39	0.54	-	-
22-Mar-92	+24	15.3	12.4	9.4	12.2	6.1	15.2	11.76	1.32	14.3	11.6	13.4	13.9	13.3	12.6	13.17	0.36	13.6	13.7	12.8	17.1	14.2	15.0	14.39	0.55	-	-
29-Mar-92	+25	15.2	13.8	9.8	12.6	5.8	14.5	11.92	1.33	14.2	12.2	13.3	13.2	13.5	13.1	13.24	0.24	14.2	14.8	13.3	15.6	11.8	15.3	14.16	0.53	2.54	0.2804
05-Apr-92	+26	14.8	12.6	9.0	12.0	5.9	12.0	11.05	1.17	13.9	12.0	12.9	12.7	13.3	12.5	12.88	0.23	13.3	14.9	14.6	14.5	13.0	15.3	14.25	0.33	-	-
12-Apr-92	+27	13.6	12.6	9.8	11.3	6.3	16.1	11.62	1.25	13.2	12.2	14.6	14.1	11.0	13.7	13.13	0.49	14.4	14.3	12.5	16.3	13.8	13.4	14.10	0.48	-	-
19-Apr-92	+28	13.4	11.8	10.5	10.9	5.4	14.4	11.05	1.17	13.3	12.6	13.9	13.7	13.7	13.6	13.46	0.17	13.7	14.8	12.9	15.3	13.7	13.5	13.98	0.33	-	-
26-Apr-92	+29	12.9	11.0	10.2	10.4	5.5	14.7	10.78	1.16	13.3	12.6	12.9	13.9	13.9	13.1	13.25	0.19	12.0	15.3	13.4	15.5	14.3	13.8	14.03	0.49	5.86	0.0535
03-May-92	**+30**	13.3	10.8	10.1	10.1	5.9	13.0	10.51	1.00	13.0	12.2	15.3	14.3	14.9	13.8	13.92	0.43	14.6	15.3	12.9	14.9	14.3	13.9	14.30	0.31	8.41	0.0149
10-May-92	+31	13.7	11.3	10.9	10.9	5.3	14.8	11.14	1.22	13.7	12.0	13.3	15.0	14.6	13.6	13.69	0.40	13.3	16.1	13.3	16.0	14.6	14.2	14.58	0.46	4.62	0.0992
17-May-92	+32	14.3	11.3	10.3	11.5	5.9	14.6	11.31	1.18	14.1	13.1	12.6	14.8	15.6	14.7	14.14	0.42	14.4	15.8	12.3	15.7	14.2	15.0	14.56	0.48	-	-
24-May-92	+33	13.9	7.0	11.7	10.8	5.2	16.0	10.76	1.52	13.8	11.6	14.0	14.1	15.4	13.8	13.81	0.46	15.8	12.8	13.7	16.1	14.9	14.3	14.60	0.46	-	-
31-May-92	+34	14.2	**	11.8	10.8	6.1	14.8	11.52	1.38	14.2	12.0	12.7	14.2	15.5	14.7	13.87	0.48	15.3	13.6	11.7	15.8	13.4	15.0	14.13	0.58	-	-
07-May-92	+35	15.0	**	12.3	11.0	6.6	14.7	11.89	1.36	14.7	12.5	14.0	14.9	14.4	13.5	13.99	0.33	15.3	15.6	14.3	15.7	13.5	14.7	14.84	0.32	3.93	0.1401

Statistics = results Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;
**** = animal dead; the weeks when the differences between the groups were significant, are printed in bold faces.**

Appendix Table 8.3.7: Packed cell volumes (l/l) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

Date	WPI	Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
		31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	0.28	0.24	0.23	0.23	0.25	0.28	0.25	0.01	0.24	0.21	0.24	0.27	0.26	0.24	0.24	0.01	0.27	0.23	0.26	0.27	0.27	0.28	0.26	0.01	2.1	0.3435
08-Sep-91	-4	0.25	0.25	0.22	0.24	0.26	0.26	0.25	0.01	0.25	0.22	0.24	0.26	0.25	0.23	0.24	0.01	0.26	0.22	0.25	0.26	0.25	0.29	0.26	0.01	-	-
15-Sep-91	-3	0.25	0.24	0.22	0.25	0.27	0.25	0.25	0.01	0.24	0.23	0.26	0.27	0.28	0.24	0.25	0.01	0.27	0.21	0.26	0.25	0.25	0.29	0.26	0.01	-	-
22-Sep-91	-2	0.25	0.26	0.21	0.25	0.25	0.28	0.25	0.01	0.26	0.23	0.25	0.27	0.28	0.25	0.26	0.01	0.28	0.22	0.26	0.24	0.26	0.30	0.26	0.01	-	-
29-Sep-91	-1	0.25	0.25	0.21	0.25	0.27	0.27	0.25	0.01	0.26	0.23	0.28	0.28	0.27	0.25	0.26	0.01	0.27	0.21	0.25	0.26	0.27	0.28	0.26	0.01	-	-
06-Oct-91	0	0.26	0.24	0.22	0.24	0.28	0.26	0.25	0.01	0.28	0.23	0.29	0.26	0.26	0.26	0.26	0.01	0.26	0.22	0.27	0.28	0.28	0.27	0.26	0.01	1.9	0.3949
13-Oct-91	+1	0.30	0.23	0.25	0.25	0.25	0.30	0.26	0.01	0.26	0.23	0.28	0.26	0.30	0.27	0.27	0.01	0.25	0.24	0.24	0.26	0.30	0.25	0.26	0.01	-	-
20-Oct-91	+2	0.28	0.22	0.25	0.22	0.26	0.26	0.25	0.01	0.29	0.22	0.25	0.22	0.29	0.25	0.25	0.01	0.27	0.22	0.25	0.25	0.30	0.25	0.26	0.01	-	-
27-Oct-91	+3	0.28	0.24	0.25	0.24	0.26	0.27	0.26	0.01	0.29	0.26	0.27	0.23	0.29	0.25	0.27	0.01	0.26	0.20	0.28	0.27	0.33	0.25	0.27	0.02	-	-
03-Nov-91	+4	0.28	0.23	0.28	0.23	0.25	0.30	0.26	0.01	0.31	0.28	0.30	0.25	0.32	0.27	0.29	0.01	0.25	0.22	0.30	0.25	0.30	0.25	0.26	0.01	-	-
10-Nov-91	+5	0.27	0.24	0.28	0.28	0.27	0.30	0.27	0.01	0.30	0.28	0.32	0.26	0.31	0.30	0.30	0.01	0.30	0.23	0.28	0.28	0.30	0.23	0.27	0.01	3.3	0.1815
17-Nov-91	+6	0.29	0.26	0.28	0.24	0.26	0.28	0.27	0.01	0.28	0.25	0.28	0.27	0.30	0.27	0.28	0.01	0.28	0.22	0.28	0.26	0.27	0.25	0.26	0.01	-	-
24-Nov-91	+7	0.30	0.23	0.29	0.25	0.26	0.32	0.28	0.01	0.30	0.25	0.28	0.26	0.33	0.26	0.28	0.01	0.29	0.23	0.25	0.27	0.30	0.28	0.27	0.01	-	-
01-Dec-91	+8	0.30	0.24	0.28	0.26	0.28	0.32	0.28	0.01	0.30	0.26	0.31	0.30	0.35	0.26	0.30	0.01	0.27	0.25	0.29	0.27	0.27	0.28	0.27	0.00	-	-
08-Dec-91	+9	0.31	0.25	0.30	0.31	0.28	0.34	0.30	0.01	0.29	0.26	0.30	0.25	0.34	0.29	0.29	0.01	0.33	0.27	0.30	0.32	0.28	0.24	0.29	0.01	-	-
15-Dec-91	+10	0.32	0.25	0.31	0.28	0.25	0.31	0.29	0.01	0.29	0.28	0.32	0.27	0.36	0.28	0.30	0.01	0.30	0.31	0.32	0.30	0.30	0.28	0.30	0.00	0.6	0.7423
22-Dec-91	+11	0.31	0.29	0.30	0.30	0.22	0.34	0.29	0.01	0.28	0.27	0.32	0.30	0.36	0.30	0.31	0.01	0.33	0.30	0.31	0.35	0.32	0.30	0.32	0.01	-	-
29-Dec-91	+12	0.28	0.28	0.35	0.25	0.15	0.30	0.27	0.02	0.28	0.26	0.33	0.27	0.31	0.27	0.29	0.01	0.29	0.29	0.32	0.31	0.30	0.30	0.30	0.00	2.5	0.2935
05-Jan-92	**+13**	0.29	0.21	0.21	0.23	0.13	0.30	0.23	0.02	0.28	0.28	0.31	0.29	0.31	0.27	0.29	0.01	0.29	0.30	0.28	0.31	0.30	0.32	0.30	0.01	7.4	0.0245
12-Jan-92	**+14**	0.30	0.23	0.21	0.24	0.16	0.30	0.24	0.02	0.29	0.28	0.32	0.30	0.32	0.26	0.30	0.01	0.30	0.31	0.28	0.32	0.28	0.30	0.30	0.01	-	-
19-Jan-92	**+15**	0.27	0.23	0.23	0.22	0.18	0.24	0.23	0.01	0.26	0.26	0.31	0.28	0.31	0.27	0.28	0.01	0.30	0.29	0.28	0.30	0.28	0.30	0.29	0.00	10.4	0.0054
26-Jan-92	**+16**	0.28	0.24	0.25	0.23	0.18	0.26	0.24	0.01	0.28	0.26	0.31	0.28	0.33	0.27	0.29	0.01	0.30	0.28	0.28	0.30	0.30	0.30	0.29	0.00	-	-
02-Feb-92	**+17**	0.30	0.27	0.25	0.25	0.19	0.30	0.26	0.02	0.32	0.26	0.35	0.28	0.34	0.30	0.31	0.01	0.31	0.32	0.30	0.37	0.28	0.30	0.31	0.01	-	-
09-Feb-92	**+18**	0.33	0.24	0.25	0.22	0.17	0.28	0.25	0.02	0.35	0.29	0.34	0.29	0.33	0.30	0.32	0.01	0.31	0.30	0.29	0.34	0.29	0.30	0.31	0.01	-	-
16-Feb-92	**+19**	0.29	0.26	0.28	0.25	0.17	0.21	0.24	0.02	0.29	0.28	0.30	0.28	0.33	0.29	0.30	0.01	0.32	0.30	0.29	0.31	0.30	0.32	0.31	0.00	-	-
23-Feb-92	**+20**	0.28	0.27	0.27	0.24	0.16	0.28	0.25	0.02	0.32	0.29	0.31	0.28	0.28	0.29	0.30	0.01	0.29	0.29	0.30	0.30	0.29	0.31	0.30	0.00	10.7	0.0046
01-Mar-92	**+21**	0.28	0.26	0.22	0.26	0.17	0.27	0.24	0.02	0.28	0.29	0.30	0.28	0.30	0.29	0.29	0.00	0.30	0.30	0.31	0.30	0.29	0.30	0.30	0.00	-	-
08-Mar-92	**+22**	0.27	0.25	0.22	0.23	0.15	0.27	0.23	0.02	0.28	0.25	0.30	0.28	0.29	0.30	0.28	0.01	0.29	0.28	0.30	0.30	0.29	0.29	0.29	0.00	-	-
15-Mar-92	**+23**	0.27	0.26	0.25	0.25	0.15	0.28	0.24	0.02	0.26	0.27	0.31	0.29	0.29	0.25	0.28	0.01	0.29	0.28	0.30	0.29	0.28	0.28	0.29	0.00	-	-
22-Mar-92	**+24**	0.27	0.24	0.22	0.24	0.12	0.28	0.23	0.02	0.27	0.27	0.30	0.29	0.28	0.26	0.28	0.01	0.30	0.28	0.30	0.29	0.30	0.28	0.29	0.00	-	-
29-Mar-92	**+25**	0.29	0.29	0.23	0.24	0.11	0.28	0.24	0.03	0.28	0.27	0.31	0.29	0.29	0.27	0.29	0.01	0.31	0.31	0.29	0.30	0.29	0.30	0.30	0.00	7.9	0.0192
05-Apr-92	**+26**	0.29	0.23	0.22	0.23	0.11	0.25	0.22	0.02	0.27	0.25	0.30	0.26	0.28	0.28	0.27	0.01	0.30	0.30	0.29	0.29	0.30	0.30	0.30	0.00	-	-
12-Apr-92	**+27**	0.27	0.25	0.25	0.23	0.11	0.30	0.24	0.02	0.27	0.28	0.32	0.26	0.25	0.30	0.28	0.01	0.30	0.31	0.30	0.30	0.30	0.31	0.30	0.00	-	-
19-Apr-92	**+28**	0.25	0.23	0.23	0.21	0.09	0.26	0.21	0.02	0.26	0.26	0.29	0.28	0.31	0.28	0.28	0.01	0.31	0.32	0.29	0.29	0.31	0.30	0.30	0.00	-	-
26-Apr-92	**+29**	0.24	0.21	0.22	0.19	0.10	0.27	0.21	0.02	0.26	0.25	0.28	0.24	0.26	0.25	0.26	0.01	0.31	0.31	0.29	0.28	0.29	0.29	0.30	0.00	-	-
03-May-92	**+30**	0.26	0.23	0.23	0.20	0.11	0.30	0.22	0.02	0.28	0.26	0.33	0.26	0.26	0.30	0.28	0.01	0.30	0.30	0.26	0.30	0.29	0.28	0.29	0.01	6.9	0.0489
10-May-92	**+31**	0.26	0.27	0.25	0.21	0.09	0.30	0.23	0.03	0.26	0.26	0.30	0.31	0.31	0.29	0.29	0.01	0.28	0.31	0.27	0.28	0.29	0.29	0.29	0.01	-	-
17-May-92	**+32**	0.27	0.21	0.25	0.23	0.12	0.27	0.23	0.02	0.28	0.27	0.27	0.29	0.31	0.30	0.29	0.01	0.29	0.31	0.28	0.30	0.28	0.29	0.29	0.00	-	-
24-May-92	**+33**	0.27	0.22	0.26	0.21	0.10	0.28	0.22	0.02	0.28	0.25	0.30	0.28	0.32	0.30	0.29	0.01	0.33	0.30	0.29	0.30	0.37	0.29	0.31	0.01	-	-
31-May-92	**+34**	0.24	**	0.21	0.26	0.09	0.24	0.21	0.03	0.29	0.27	0.26	0.30	0.29	0.28	0.28	0.01	0.29	0.32	0.30	0.29	0.32	0.30	0.30	0.01	-	-
07-Jun-92	**+35**	0.28	**	0.23	0.20	0.10	0.27	0.22	0.03	0.27	0.25	0.30	0.27	0.29	0.26	0.27	0.01	0.32	0.32	0.29	0.30	0.32	0.29	0.31	0.01	10.3	0.0059

Statistics = results Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;
**** = animal dead; the weeks when the differences between the groups were significant, are printed in bold faces.**

Appendix Table 8.3.8: Haemoglobin concentrations (g/l) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
Date	WPI	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	98	98	86	85	89	108	94.1	3.4	104	93	85	93	84	85	90.5	2.9	93	85	113	98	98	85	95.3	3.9	1.3	0.5197
08-Sep-91	-4	99	95	90	86	86	100	92.6	2.4	100	88	84	90	83	84	88.1	2.4	91	83	103	93	93	86	91.4	2.6	-	-
15-Sep-91	-3	102	98	93	90	90	116	98.1	3.6	108	97	87	93	85	86	92.6	3.3	93	85	109	99	97	90	95.4	3.1	-	-
22-Sep-91	-2	101	98	85	85	85	102	92.6	3.1	104	93	85	95	88	85	91.7	2.7	93	84	103	93	93	85	91.7	2.6	-	-
29-Sep-91	-1	99	93	83	83	89	115	93.7	4.6	100	89	93	94	84	85	90.8	2.3	91	85	113	99	98	94	96.7	3.5	-	-
06-Oct-91	0	102	96	90	92	93	108	96.8	2.5	104	88	100	90	90	99	95.1	2.5	93	85	117	96	115	98	100.5	4.6	0.6	0.7495
13-Oct-91	+1	103	87	97	85	97	105	95.7	3.0	103	77	101	85	97	93	92.5	3.6	93	80	105	89	116	99	96.9	4.7	-	-
20-Oct-91	+2	114	89	98	90	92	98	96.9	3.5	106	74	98	90	123	90	96.7	6.2	98	82	120	98	123	98	103.1	5.8	-	-
27-Oct-91	+3	109	85	93	90	97	126	100.0	5.7	113	94	92	93	112	96	100.1	3.6	91	81	125	99	115	93	100.6	6.1	-	-
03-Nov-91	+4	114	85	125	97	92	95	101.1	5.6	114	99	107	99	116	111	107.6	2.8	96	86	117	100	115	93	101.0	4.7	-	-
10-Nov-91	+5	104	88	95	95	97	95	95.7	1.8	104	95	110	89	114	103	102.4	3.4	105	84	101	98	105	85	96.2	3.6	2.0	0.3612
17-Nov-91	+6	102	95	103	89	96	101	97.8	2.0	109	96	116	105	111	101	106.6	2.6	106	81	110	94	104	88	97.1	4.3	-	-
24-Nov-91	+7	101	90	94	84	93	113	95.9	3.7	103	96	94	104	111	108	102.7	2.5	104	88	106	93	105	96	98.8	2.9	-	-
01-Dec-91	+8	106	84	97	90	97	109	96.9	3.5	106	92	87	100	126	90	100.0	5.3	102	87	105	91	102	91	96.1	2.9	-	-
08-Dec-91	+9	104	96	104	85	87	118	99.1	4.7	94	91	103	104	125	94	101.8	4.7	101	85	103	100	110	96	99.1	3.1	-	-
15-Dec-91	+10	110	79	105	99	83	101	96.3	4.7	93	95	118	93	126	98	103.7	5.4	108	101	110	104	106	93	103.8	2.3	1.1	0.5854
22-Dec-91	+11	115	99	91	96	78	118	99.4	5.6	105	98	116	103	131	110	110.5	4.4	111	109	117	110	104	99	108.4	2.4	1.8	0.3968
29-Dec-91	+12	112	102	91	95	62	104	94.3	6.5	100	97	101	99	122	99	103.1	3.5	104	107	108	112	102	99	105.5	1.6	2.8	0.2472
05-Jan-92	+13	115	81	96	95	56	108	91.7	7.8	98	106	106	105	108	95	102.9	2.0	102	104	106	112	108	105	106.3	1.3	1.7	0.4251
12-Jan-92	+14	107	73	64	79	55	106	80.6	8.1	91	93	110	107	117	95	102.3	4.0	109	104	110	113	106	106	107.9	1.2	5.6	0.0601
19-Jan-92	**+15**	94	78	78	68	59	89	77.7	4.8	99	89	113	100	98	85	97.4	3.5	107	106	110	105	105	101	105.7	1.1	11.5	0.0032
26-Jan-92	**+16**	91	79	84	64	59	87	77.4	4.8	95	93	93	89	102	89	93.5	1.7	106	103	104	118	109	105	107.5	2.0	-	-
02-Feb-92	**+17**	111	98	86	90	68	108	93.4	5.9	108	101	132	99	123	86	108.3	6.2	106	119	105	122	107	113	112.0	2.7	-	-
09-Feb-92	**+18**	117	90	83	81	61	95	87.9	6.8	112	106	112	100	111	104	107.6	1.8	102	116	109	119	103	104	108.9	2.7	-	-
16-Feb-92	**+19**	97	82	83	78	53	99	82.1	6.2	98	99	111	95	112	102	102.8	2.7	109	107	108	110	107	110	108.6	0.5	-	-
23-Feb-92	**+20**	96	94	88	85	56	102	86.8	6.0	106	102	101	112	97	89	101.0	3.0	110	118	108	116	104	106	110.2	2.0	10.9	0.0043
01-Mar-92	**+21**	108	97	82	89	61	97	88.9	6.0	114	101	112	101	92	108	104.7	3.0	108	104	104	108	123	114	110.2	2.6	-	-
08-Mar-92	**+22**	101	87	71	75	50	88	78.8	6.5	102	93	112	102	106	103	103.0	2.3	109	112	124	118	103	106	112.0	2.9	-	-
15-Mar-92	**+23**	98	99	81	79	42	92	81.9	7.9	88	83	99	98	101	97	94.4	2.6	118	106	105	124	105	112	111.5	2.9	-	-
22-Mar-92	**+24**	99	85	72	82	43	99	80.0	7.7	99	95	92	89	95	92	93.6	1.3	117	111	104	111	101	114	109.6	2.2	-	-
29-Mar-92	**+25**	96	97	79	89	36	94	81.8	8.8	91	91	100	98	95	94	95.0	1.3	111	106	114	121	100	111	110.5	2.6	11.8	0.0027
05-Apr-92	**+26**	95	85	72	78	35	76	73.6	7.7	95	91	100	82	98	98	94.0	2.4	111	105	109	111	101	104	107.0	1.6	-	-
12-Apr-92	**+27**	95	88	79	76	37	112	81.0	9.4	92	86	108	98	83	103	95.2	3.6	108	101	106	117	117	105	108.9	2.4	-	-
19-Apr-92	**+28**	93	85	80	78	34	103	78.7	8.9	97	94	107	94	98	101	98.4	1.9	107	108	106	119	103	101	107.6	2.4	-	-
26-Apr-92	**+29**	91	84	91	73	39	96	79.0	8.0	98	92	101	99	93	116	99.7	3.2	106	113	113	102	102	109	107.6	1.8	-	-
03-May-92	**+30**	88	78	77	74	38	82	72.6	6.6	90	91	100	100	107	109	99.5	2.9	107	103	104	107	113	111	107.7	1.4	12.8	0.0016
10-May-92	**+31**	100	85	95	78	35	102	82.3	9.3	98	88	98	104	107	107	100.1	2.7	102	110	105	111	115	118	110.3	2.2	-	-
17-May-92	**+32**	100	88	88	80	44	112	85.2	8.6	102	99	97	108	104	105	102.5	1.5	107	119	111	117	111	115	113.4	1.7	-	-
24-May-92	**+33**	98	78	90	77	36	110	81.6	9.6	106	96	104	110	123	113	108.6	3.4	118	116	124	111	113	115	116.0	1.7	-	-
31-May-92	**+34**	93	**	89	74	36	96	77.6	9.9	89	88	94	104	115	106	99.3	4.0	102	113	116	102	117	104	109.1	2.6	-	-
07-Jun-92	**+35**	97	**	90	75	40	92	78.9	9.3	98	94	102	105	113	97	101.6	2.6	126	108	130	127	108	101	116.7	4.7	11.5	0.0031

Statistics = results of Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;
**** = animal dead; the weeks when the differences between the groups were significant are printed in bold faces.**

Appendix Table 8.3.9: Mean cell volumes (fl) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
Date	WPI	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	23.7	19.5	24.2	19.5	20.5	22.8	21.70	0.79	20.9	21.4	22.9	23.7	23.6	22.3	22.46	0.43	22.1	21.7	24.8	23.9	25.0	23.0	23.41	0.51	2.7	0.2557
08-Sep-91	-4	20.2	21.2	22.4	20.5	20.8	21.8	21.16	0.32	20.5	23.2	23.1	23.2	23.1	23.5	22.76	0.42	23.6	20.0	23.4	22.6	23.1	22.6	22.56	0.49	-	-
15-Sep-91	-3	19.4	20.3	22.9	20.9	22.4	22.2	21.35	0.51	21.7	24.5	22.4	23.4	21.2	24.2	22.91	0.50	23.9	20.0	24.9	21.4	23.5	25.4	23.18	0.78	-	-
22-Sep-91	-2	20.5	20.5	22.1	21.4	21.6	22.2	21.38	0.28	21.2	24.7	23.8	23.7	21.2	25.5	23.35	0.67	22.2	20.4	25.3	20.6	23.3	23.8	22.59	0.71	-	-
29-Sep-91	-1	21.2	22.0	20.6	22.5	21.3	20.3	21.31	0.32	22.4	25.0	23.0	22.3	21.5	24.6	23.15	0.51	22.3	19.7	21.0	22.1	25.4	25.9	22.73	0.92	-	-
06-Oct-91	0	21.6	20.4	21.1	22.5	20.4	21.1	21.17	0.30	21.3	21.2	24.2	21.1	22.1	25.3	22.53	0.67	24.2	20.6	24.3	23.4	26.8	24.3	23.93	0.75	5.9	0.0531
13-Oct-91	+1	21.0	20.6	22.4	21.2	19.4	22.2	21.14	0.41	19.0	21.7	23.2	23.0	22.7	25.4	22.49	0.78	21.7	23.1	20.8	22.6	29.9	21.9	23.32	1.23	-	-
20-Oct-91	+2	20.4	20.3	23.9	20.0	18.5	17.8	20.15	0.79	20.4	18.3	20.8	18.3	23.4	19.8	20.16	0.70	23.1	20.7	20.9	19.4	19.7	20.6	20.72	0.48	-	-
27-Oct-91	+3	20.8	19.7	20.9	18.7	19.0	17.3	19.41	0.51	18.6	20.4	21.9	17.5	22.5	19.9	20.14	0.71	20.5	18.0	21.6	18.8	19.6	20.3	19.81	0.47	-	-
03-Nov-91	+4	19.8	21.9	23.0	18.2	19.5	20.2	20.42	0.64	20.4	21.6	22.4	19.7	20.1	19.6	20.63	0.42	22.7	19.1	23.5	17.2	20.3	21.9	20.77	0.89	-	-
10-Nov-91	+5	21.3	21.6	21.2	19.0	20.2	19.6	20.46	0.39	20.2	20.8	22.3	20.5	22.2	21.2	21.21	0.34	23.2	18.5	21.3	19.5	20.1	20.2	20.46	0.60	1.7	0.4201
17-Nov-91	+6	21.0	21.3	22.5	19.9	19.8	19.9	20.73	0.40	20.9	20.4	22.2	20.1	22.2	20.8	21.10	0.33	21.3	18.2	22.0	20.1	18.8	21.3	20.28	0.57	-	-
24-Nov-91	+7	20.3	18.4	21.3	18.2	19.5	18.0	19.29	0.49	20.0	19.9	21.3	19.0	23.7	20.7	20.79	0.61	20.5	18.7	20.6	21.4	20.2	21.3	20.47	0.37	-	-
01-Dec-91	+8	20.0	21.6	22.8	20.3	19.9	20.5	20.83	0.42	20.9	21.7	26.5	19.9	23.3	20.1	22.05	0.92	22.6	18.8	21.7	19.5	17.9	23.0	20.58	0.80	-	-
08-Dec-91	+9	22.7	21.9	24.7	22.1	23.2	21.6	22.70	0.42	20.0	21.8	23.0	20.8	22.7	23.9	22.05	0.55	25.4	20.0	22.7	20.8	20.1	20.8	21.63	0.77	-	-
15-Dec-91	+10	19.8	20.6	23.1	18.3	20.9	21.8	20.75	0.61	22.3	21.7	22.8	21.0	22.8	22.1	22.12	0.26	22.3	20.5	22.7	19.5	19.9	25.1	21.68	0.78	2.2	0.3270
22-Dec-91	+11	18.2	21.6	22.7	19.8	19.6	19.4	20.22	0.61	19.0	20.7	20.9	21.1	21.8	22.7	21.04	0.46	25.0	19.6	22.7	21.5	21.3	23.7	22.31	0.71	-	-
29-Dec-91	+12	18.2	19.4	31.0	18.7	18.9	19.5	20.96	1.84	20.8	20.3	21.2	19.4	19.6	20.4	20.28	0.26	22.3	19.3	24.2	18.7	19.8	22.4	21.11	0.80	-	-
05-Jan-92	+13	19.0	19.8	20.6	18.6	17.4	18.8	19.03	0.41	20.7	21.7	20.8	19.8	20.6	20.1	20.60	0.25	21.1	18.8	22.2	18.5	20.2	22.2	20.51	0.61	-	-
12-Jan-92	+14	19.3	21.0	22.5	19.3	20.1	18.6	20.11	0.54	22.3	21.3	22.1	20.2	20.4	19.3	20.93	0.44	24.6	19.6	22.4	18.5	19.5	20.6	20.89	0.84	-	-
19-Jan-92	+15	16.4	19.4	22.9	20.1	19.5	18.0	19.37	0.81	17.8	20.4	20.3	18.3	28.5	22.0	21.20	1.45	20.9	17.7	20.8	17.3	17.9	20.2	19.13	0.62	1.5	0.4758
26-Jan-92	+16	18.2	19.6	23.5	19.7	20.2	18.9	20.03	0.69	20.0	21.9	23.4	19.8	22.4	19.3	21.15	0.62	21.0	17.9	20.8	17.9	19.7	20.9	19.71	0.55	-	-
02-Feb-92	+17	19.2	21.8	23.2	21.3	22.3	19.7	21.26	0.57	21.9	20.7	21.2	20.6	20.2	28.3	22.15	1.14	24.8	18.9	20.6	18.7	19.5	20.9	20.56	0.84	-	-
09-Feb-92	+18	20.8	20.1	23.7	20.5	21.7	18.8	20.93	0.61	23.3	22.5	22.8	20.4	21.9	23.8	22.44	0.45	22.9	18.5	21.5	19.1	19.7	20.4	20.34	0.60	-	-
16-Feb-92	+19	18.4	19.0	21.1	19.7	21.1	12.3	18.62	1.21	19.0	18.9	18.3	20.0	20.1	19.4	19.26	0.26	20.4	17.9	25.0	18.8	18.4	19.0	19.93	0.98	-	-
23-Feb-92	+20	15.9	19.9	22.6	19.4	20.4	17.6	19.28	0.87	21.2	21.1	22.4	19.2	19.8	23.9	21.28	0.64	21.4	18.4	22.5	17.7	20.1	21.0	20.19	0.68	2.0	0.3722
01-Mar-92	+21	18.8	19.2	20.9	20.7	26.5	18.0	20.67	1.14	19.3	23.0	20.6	20.9	23.8	22.5	21.70	0.63	22.9	19.7	23.8	18.1	19.0	20.3	20.64	0.84	-	-
08-Mar-92	+22	18.5	19.4	19.5	19.5	20.5	19.7	19.50	0.24	19.6	19.5	21.1	21.4	21.0	22.8	20.88	0.46	21.6	21.1	21.3	18.0	19.9	19.2	20.20	0.52	-	-
15-Mar-92	+23	19.1	19.7	22.5	20.7	20.5	18.2	20.11	0.56	19.4	23.1	22.1	20.3	20.4	21.7	21.17	0.51	22.8	19.4	23.0	17.6	18.0	19.9	20.11	0.86	-	-
22-Mar-92	+24	17.6	19.4	23.4	19.8	19.6	18.4	19.71	0.75	18.8	23.2	22.5	20.9	21.1	20.7	21.20	0.57	22.1	20.5	23.4	17.0	21.2	18.7	20.46	0.86	-	-
29-Mar-92	+25	19.0	21.1	23.6	19.1	19.0	19.4	20.20	0.69	19.8	22.2	23.3	22.1	21.5	20.6	21.56	0.46	21.8	20.9	21.8	19.3	24.7	19.7	21.35	0.72	3.4	0.1817
05-Apr-92	+26	19.6	18.3	24.4	19.1	18.8	20.8	20.16	0.83	19.5	20.8	23.3	20.4	21.1	22.4	21.25	0.51	22.5	20.1	19.9	20.1	23.1	19.7	20.90	0.56	-	-
12-Apr-92	+27	19.9	19.8	25.4	20.4	17.5	18.6	20.27	1.02	20.5	22.9	22.0	18.5	22.7	21.9	21.40	0.62	20.9	21.7	24.1	18.4	21.7	23.2	21.66	0.73	-	-
19-Apr-92	+28	18.6	19.5	22.0	19.3	16.7	18.1	19.04	0.66	19.6	20.6	20.9	20.4	22.6	20.6	20.79	0.37	22.7	21.7	22.5	19.0	22.6	22.2	21.75	0.53	-	-
26-Apr-92	+29	18.6	19.1	21.5	18.3	18.3	18.4	19.04	0.47	19.6	19.8	21.8	17.3	18.8	19.1	19.40	0.55	25.9	20.2	21.7	18.1	20.3	21.1	21.21	0.98	-	-
03-May-92	+30	19.6	21.3	22.8	19.8	18.8	23.2	20.91	0.67	21.5	21.3	21.6	18.2	17.4	21.7	20.30	0.73	20.5	19.7	20.2	20.2	20.3	20.1	20.17	0.11	0.5	0.8063
10-May-92	+31	19.0	23.9	23.0	19.3	16.9	20.3	20.39	0.98	19.0	21.7	22.6	20.7	21.3	21.3	21.09	0.46	21.1	19.3	20.3	17.5	19.9	20.4	19.74	0.47	-	-
17-May-92	+32	18.9	18.6	24.3	20.1	20.4	18.5	20.12	0.81	19.8	20.7	21.5	19.6	19.9	20.4	20.32	0.26	20.1	19.6	22.8	19.2	19.7	19.4	20.12	0.50	-	-
24-May-92	+33	19.4	31.7	22.3	19.4	19.1	17.5	21.56	1.93	20.2	21.5	21.4	19.8	20.8	21.7	20.90	0.28	20.9	23.4	21.1	18.7	24.8	20.3	21.53	0.82	-	-
31-May-92	+34	17.0	**	21.9	24.0	19.8	18.2	20.16	1.13	20.4	22.6	20.4	21.1	18.8	19.1	20.40	0.52	19.0	23.6	25.8	18.3	23.8	20.0	21.74	1.14	-	-
07-Jun-92	+35	18.7	**	22.8	22.3	20.1	18.4	20.45	0.80	18.3	19.9	21.5	18.2	20.2	19.3	19.57	0.46	20.9	20.5	20.4	19.2	23.7	19.8	20.72	0.59	1.9	0.3816

Statistics = results of Mann Whitney test: U = U statistic; P = significance (probability); - = analysis not done; ** = animal dead, the weeks when the faecal egg counts in the groups A and B were significantly different are printed in bold faces.

Appendix Table 8.3.10: Mean Cell Haemoglobin (pg) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

Date	WPI	Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
		31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	8.3	8.0	9.1	7.2	7.3	8.8	8.11	0.29	9.0	9.5	8.1	8.1	7.6	8.9	8.54	0.26	8.3	8.0	10.8	8.7	9.0	7.6	8.74	0.41	1.1	0.5857
08-Sep-91	-4	8.0	8.0	9.2	7.3	6.9	8.4	7.97	0.30	8.2	9.2	8.1	8.1	7.7	8.6	8.30	0.20	8.3	7.5	9.6	8.1	8.6	7.9	8.33	0.27	-	-
15-Sep-91	-3	7.9	8.3	9.7	7.5	7.5	10.2	8.52	0.43	9.8	10.3	8.5	8.0	8.3	8.6	8.92	0.33	8.2	8.1	10.4	8.5	9.1	7.8	8.69	0.35	-	-
22-Sep-91	-2	8.3	7.7	8.9	7.3	7.4	8.1	7.93	0.23	8.5	10.0	8.1	8.4	7.9	8.7	8.58	0.27	9.0	7.8	10.0	8.0	8.3	7.6	8.44	0.34	-	-
29-Sep-91	-1	8.4	8.2	8.1	7.5	7.0	8.7	7.97	0.23	8.6	9.7	9.0	9.2	8.2	8.4	8.84	0.20	8.9	8.0	9.5	8.4	9.2	8.7	8.78	0.20	-	-
06-Oct-91	0	7.7	8.1	8.6	7.9	6.8	7.9	7.83	0.23	7.9	8.1	8.3	7.3	8.3	9.6	8.27	0.28	8.7	8.0	10.5	8.0	11.0	8.8	9.15	0.48	5.3	0.0693
13-Oct-91	+1	7.2	7.8	8.7	7.2	7.5	7.8	7.71	0.21	7.5	7.3	8.3	7.5	9.6	8.7	8.15	0.33	8.0	7.7	9.1	7.7	11.5	8.6	8.79	0.54	-	-
20-Oct-91	+2	7.5	7.4	9.4	8.2	6.6	6.7	7.62	0.39	7.5	6.1	8.2	7.5	9.9	7.1	7.70	0.47	8.4	7.7	10.0	7.6	8.0	8.1	8.30	0.33	-	-
27-Oct-91	+3	7.0	6.6	7.7	7.0	7.1	8.1	7.26	0.21	7.2	7.4	7.4	7.1	8.7	7.7	7.59	0.22	7.2	7.2	9.6	6.9	6.8	7.6	7.56	0.39	-	-
03-Nov-91	+4	7.6	6.9	10.2	7.7	7.2	6.4	7.67	0.50	7.5	7.6	8.0	7.8	7.3	8.0	7.70	0.11	8.7	7.4	9.2	6.9	7.8	8.2	8.01	0.31	-	-
10-Nov-91	+5	7.0	7.2	7.4	7.5	7.2	6.7	7.18	0.11	7.0	7.0	7.7	7.0	8.2	7.3	7.36	0.18	8.1	6.7	7.6	6.9	7.0	7.4	7.30	0.20	0.3	0.8456
17-Nov-91	+6	7.4	7.8	8.3	7.4	7.3	7.2	7.56	0.15	8.1	7.9	9.2	7.8	8.2	7.8	8.18	0.19	8.0	6.7	8.6	7.2	7.3	7.5	7.56	0.25	-	-
24-Nov-91	+7	6.9	7.2	6.9	6.2	6.9	6.3	6.74	0.15	6.9	7.6	7.2	7.6	8.0	8.6	7.65	0.23	7.4	7.1	8.8	7.4	7.1	7.3	7.51	0.24	-	-
01-Dec-91	+8	7.1	7.5	7.9	7.0	6.9	7.0	7.21	0.15	7.4	7.7	7.4	6.7	8.3	6.9	7.40	0.22	8.5	6.5	7.8	6.6	6.8	7.4	7.27	0.30	-	-
08-Dec-91	+9	7.6	8.4	8.6	6.1	7.2	7.5	7.56	0.34	6.4	7.7	7.9	8.7	8.4	7.7	7.79	0.29	7.8	6.3	7.8	6.5	7.9	8.3	7.43	0.30	-	-
15-Dec-91	+10	6.8	6.5	7.8	6.5	6.9	7.1	6.95	0.19	7.2	7.3	8.4	7.2	7.9	7.7	7.63	0.18	8.1	6.7	7.8	6.8	7.0	8.3	7.46	0.26	4.8	0.0890
22-Dec-91	+11	6.7	7.3	6.9	6.4	6.9	6.7	6.83	0.12	7.1	7.5	7.6	7.3	7.9	8.4	7.62	0.17	8.4	7.2	8.6	6.8	6.9	7.8	7.61	0.29	-	-
29-Dec-91	+12	7.3	7.1	8.1	7.1	7.8	6.8	7.35	0.18	7.4	7.5	6.5	7.2	7.7	7.4	7.30	0.16	8.0	7.1	8.1	6.7	6.8	7.4	7.37	0.23	-	-
05-Jan-92	+13	7.5	7.6	9.4	7.7	7.5	6.8	7.74	0.32	7.2	8.2	7.1	7.1	7.2	7.1	7.32	0.17	7.5	6.5	8.4	6.7	7.2	7.3	7.28	0.25	-	-
12-Jan-92	+14	6.9	6.6	6.9	6.3	6.9	6.6	6.70	0.09	7.0	7.0	7.6	7.2	7.5	7.1	7.24	0.09	8.9	6.6	8.8	6.5	7.4	7.3	7.59	0.39	-	-
19-Jan-92	+15	5.7	6.5	7.8	6.2	6.4	6.6	6.56	0.26	6.8	7.0	7.4	6.5	9.0	7.0	7.27	0.33	7.5	6.5	8.2	6.0	6.7	6.8	6.95	0.29	3.8	0.1524
26-Jan-92	+16	5.9	6.5	7.9	5.5	6.7	6.3	6.46	0.31	6.8	7.8	7.0	6.3	6.9	6.4	6.88	0.20	7.4	6.6	7.7	7.0	7.1	7.3	7.22	0.14	-	-
02-Feb-92	+17	7.1	8.0	8.0	7.7	8.0	7.1	7.62	0.16	7.4	8.1	8.0	7.3	7.3	8.1	7.70	0.15	8.5	7.0	7.2	6.2	7.4	7.9	7.36	0.29	-	-
09-Feb-92	+18	7.4	7.5	7.9	7.6	7.8	6.4	7.42	0.20	7.4	8.2	7.5	7.1	7.3	8.3	7.64	0.18	7.5	7.2	8.1	6.7	7.0	7.1	7.25	0.18	-	-
16-Feb-92	+19	6.2	6.0	6.3	6.1	6.6	5.8	6.17	0.09	6.4	6.7	6.8	6.8	6.8	6.8	6.71	0.06	6.9	6.4	9.3	6.7	6.5	6.5	7.08	0.42	-	-
24-Feb-92	+20	5.4	6.9	7.4	6.9	7.1	6.4	6.68	0.26	7.0	7.4	7.3	7.7	6.8	7.3	7.26	0.11	8.1	7.5	8.1	6.9	7.3	7.2	7.49	0.19	5.0	0.0810
01-Mar-92	+21	7.2	7.1	7.7	7.1	9.5	6.5	7.53	0.39	7.8	8.0	7.7	7.6	7.3	8.4	7.81	0.14	8.2	6.9	8.0	6.5	8.0	7.7	7.56	0.26	-	-
08-Mar-92	+22	6.9	6.7	6.3	6.4	6.9	6.4	6.60	0.10	7.1	7.3	7.8	7.8	7.7	7.8	7.59	0.12	8.1	8.5	8.8	7.1	7.0	7.1	7.76	0.30	-	-
15-Mar-92	+23	6.9	7.5	7.3	6.5	5.8	6.0	6.68	0.26	6.6	7.1	7.1	6.8	7.1	8.4	7.20	0.24	9.2	7.3	8.0	7.5	6.8	8.0	7.81	0.31	-	-
22-Mar-92	+24	6.5	6.9	7.6	6.8	7.1	6.5	6.89	0.16	6.9	8.1	6.9	6.4	7.1	7.3	7.13	0.22	8.6	8.1	8.1	6.5	7.2	7.6	7.67	0.28	-	-
29-Mar-92	+25	6.3	7.1	8.1	7.1	6.1	6.5	6.87	0.27	6.4	7.5	7.5	7.5	7.0	7.2	7.19	0.15	7.8	7.1	8.6	7.8	8.6	7.3	7.85	0.23	5.9	0.0534
05-Apr-92	+26	6.4	6.8	8.0	6.5	6.0	6.3	6.66	0.27	6.9	7.5	7.7	6.5	7.4	7.9	7.31	0.20	8.3	7.0	7.5	7.7	7.8	6.8	7.53	0.20	-	-
12-Apr-92	+27	7.0	6.9	8.0	6.8	5.8	7.0	6.90	0.26	7.0	7.1	7.4	7.0	7.5	7.5	7.26	0.10	7.5	7.1	8.5	7.2	8.5	7.9	7.76	0.23	-	-
19-Apr-92	+28	6.9	7.2	7.6	7.2	6.2	7.1	7.05	0.18	7.3	7.4	7.7	6.8	7.2	7.4	7.31	0.11	7.9	7.3	8.2	7.8	7.5	7.5	7.70	0.13	-	-
26-Apr-92	+29	7.0	7.7	8.9	7.0	7.1	6.6	7.38	0.31	7.4	7.3	7.8	7.1	6.7	8.9	7.53	0.28	8.9	7.4	8.4	6.6	7.2	7.9	7.73	0.31	-	-
03-May-92	+30	6.6	7.2	7.6	7.3	6.5	6.3	6.92	0.19	6.9	7.5	6.6	7.0	7.2	7.9	7.16	0.17	7.3	6.8	8.1	7.2	7.9	8.0	7.55	0.19	3.7	0.1582
10-May-92	+31	7.3	7.5	8.7	7.2	6.5	6.9	7.35	0.28	7.2	7.3	7.4	6.9	7.3	7.8	7.32	0.11	7.7	6.9	7.9	6.9	7.9	8.3	7.60	0.21	-	-
17-May-92	+32	7.0	7.8	8.5	6.9	7.5	7.7	7.57	0.22	7.2	7.6	7.7	7.3	6.6	7.2	7.27	0.14	7.4	7.6	9.0	7.5	7.8	7.7	7.83	0.23	-	-
24-May-92	+33	7.1	8.2	7.7	7.2	6.8	6.9	7.31	0.20	7.7	8.2	7.4	7.8	8.0	8.2	7.87	0.11	7.5	9.0	9.0	6.9	7.6	8.1	8.00	0.33	-	-
31-May-92	+34	6.6	**	7.6	6.8	6.0	6.5	6.68	0.23	6.3	7.4	7.4	7.3	7.4	7.2	7.16	0.17	6.7	8.3	10.0	6.5	8.7	6.9	7.85	0.52	-	-
07-May-92	+35	6.5	**	7.4	6.9	6.1	6.3	6.61	0.20	6.7	7.5	7.3	7.1	7.9	7.2	7.27	0.15	8.2	6.9	9.2	8.1	8.0	6.9	7.87	0.32	6.5	0.0394

Statistics = results of Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;
**** = animal dead; the weeks when the differences between the groups were significant are printed in bold faces.**

Appendix Table 8.3.11: Mean Cell Haemoglobin Concentration (g/l) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

Date	WPI	Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
		31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	351	409	375	370	354	386	374.1	8.0	433	442	354	344	321	354	374.6	18.7	345	370	434	363	362	304	363.0	15.8	0.6	0.7270
08-Sep-91	-4	397	378	409	357	332	385	376.3	10.5	399	399	349	347	332	365	365.2	10.5	350	376	411	357	371	298	360.6	13.9	-	-
15-Sep-91	-3	406	409	422	360	334	462	398.9	17.0	451	420	336	344	304	356	368.4	20.7	344	405	418	397	387	309	376.7	15.6	-	-
22-Sep-91	-2	402	378	405	340	340	364	371.3	10.7	399	403	340	353	315	340	358.4	13.2	331	381	395	387	357	283	355.8	15.8	-	-
29-Sep-91	-1	396	373	394	331	328	427	375.0	14.6	384	386	333	336	309	340	348.1	11.4	337	405	452	382	362	336	379.1	16.5	-	-
06-Oct-91	0	391	400	409	384	334	414	388.6	10.9	373	381	344	347	347	379	361.8	6.6	358	388	432	341	409	362	381.8	12.8	3.2	0.2016
13-Oct-91	+1	343	380	388	340	389	350	364.9	8.6	394	336	359	327	322	344	347.0	9.9	371	335	438	342	386	394	377.6	14.0	-	-
20-Oct-91	+2	409	403	392	409	355	377	390.9	7.9	366	334	392	409	423	360	380.6	12.4	363	371	480	392	409	392	401.3	15.5	-	-
27-Oct-91	+3	390	352	370	376	373	468	388.5	15.3	389	362	340	406	387	385	378.2	8.7	350	403	446	365	349	373	381.1	13.9	-	-
03-Nov-91	+4	405	368	346	421	369	315	370.8	14.4	366	352	358	396	364	409	374.2	8.6	382	389	391	400	383	372	386.2	3.4	-	-
10-Nov-91	+5	384	368	333	394	359	340	363.1	8.9	346	338	344	343	367	343	346.8	3.8	351	364	360	351	349	368	357.1	2.9	3.2	0.2031
17-Nov-91	+6	352	365	368	372	370	362	364.9	2.6	390	386	414	390	371	376	387.6	5.6	379	367	392	361	387	351	372.8	5.9	-	-
24-Nov-91	+7	338	390	324	337	357	353	349.9	8.6	344	383	336	399	336	417	369.2	13.1	360	380	425	344	351	344	367.4	11.7	-	-
01-Dec-91	+8	352	349	345	345	345	340	346.0	1.6	352	354	280	335	359	345	337.4	11.0	378	347	362	335	378	323	353.8	8.4	-	-
08-Dec-91	+9	337	383	348	274	309	348	333.2	14.0	323	351	343	417	368	323	354.0	13.3	307	315	343	314	391	399	344.8	15.3	-	-
15-Dec-91	+10	345	315	339	354	333	327	335.5	5.2	321	338	369	345	349	349	344.9	5.8	361	325	345	348	353	332	344.1	5.0	1.3	0.5269
22-Dec-91	+11	371	340	303	321	354	347	339.3	8.9	374	363	361	344	364	367	362.4	3.7	336	365	378	315	325	329	341.4	9.2	-	-
29-Dec-91	+12	401	363	260	379	415	346	360.8	20.6	358	372	307	368	394	365	360.7	10.8	360	369	337	360	341	331	349.8	5.7	-	-
05-Jan-92	+13	395	387	355	312	332	359	356.7	11.7	350	379	343	361	347	351	355.2	4.9	353	346	380	362	359	330	355.0	6.2	-	-
12-Jan-92	+14	358	316	306	329	343	353	334.0	7.8	315	331	343	358	367	366	346.6	7.8	363	337	392	353	378	353	362.5	7.4	-	-
19-Jan-92	+15	348	338	341	311	328	369	339.2	7.2	380	344	364	358	315	317	346.1	9.7	358	364	394	350	375	337	362.9	7.5	3.0	0.2192
26-Jan-92	+16	325	330	336	278	330	335	322.3	8.2	339	356	301	319	308	331	325.7	7.6	355	369	372	393	362	349	366.7	5.8	-	-
02-Feb-92	+17	369	364	345	360	357	358	358.9	3.0	338	390	377	354	361	287	351.3	13.5	342	372	350	329	381	376	358.6	7.8	-	-
09-Feb-92	+18	354	374	334	369	359	340	354.8	5.9	320	365	330	347	335	348	340.7	5.9	329	386	376	350	355	348	357.3	7.7	-	-
16-Feb-92	+19	334	317	297	312	312	472	340.6	24.4	337	354	371	338	339	352	348.4	4.9	340	358	373	356	356	343	354.4	4.4	-	-
23-Feb-92	+20	342	346	326	354	351	365	347.3	4.8	331	352	324	401	345	306	343.3	12.1	378	406	358	387	360	342	371.9	8.6	4.7	0.0949
01-Mar-92	+21	384	373	371	343	359	359	364.7	5.3	406	347	374	362	308	373	361.7	12.2	359	348	337	361	423	379	367.9	11.4	-	-
08-Mar-92	+22	374	349	323	326	336	326	339.0	7.3	363	373	372	363	367	344	363.7	3.9	375	401	413	393	354	367	383.8	8.3	-	-
15-Mar-92	+23	362	382	325	316	283	328	332.7	13.1	338	309	321	337	348	388	340.3	10.2	405	377	349	426	377	401	389.3	10.2	-	-
22-Mar-92	+24	366	353	326	343	362	354	350.7	5.4	367	350	305	308	338	355	337.2	9.6	390	396	346	381	338	406	376.2	10.4	4.1	0.0126
29-Mar-92	+25	331	336	343	370	323	337	339.9	6.1	326	338	322	339	328	350	333.6	3.9	357	342	394	402	347	371	368.7	9.4	8.1	0.0176
05-Apr-92	+26	328	371	329	338	319	302	331.4	8.6	353	363	332	317	349	351	344.1	6.3	371	349	376	384	336	348	360.7	7.0	4.8	0.0903
12-Apr-92	+27	350	351	314	332	332	374	342.1	7.7	342	308	338	378	333	343	340.4	8.5	358	327	353	389	389	339	359.3	9.5	-	-
19-Apr-92	+28	371	370	346	373	372	395	371.3	5.8	372	360	370	334	317	361	352.2	8.2	346	338	367	412	331	337	355.3	11.3	-	-
26-Apr-92	+29	379	401	414	383	387	357	386.9	7.3	376	370	360	410	357	464	389.4	15.4	341	365	390	366	353	376	365.0	6.3	-	-
03-May-92	+30	337	337	334	369	345	274	332.7	11.8	321	351	304	383	412	363	355.6	14.8	358	345	401	358	388	397	374.4	8.9	4.7	0.0953
10-May-92	+31	384	314	378	371	387	340	362.4	10.8	378	338	325	334	344	368	347.9	7.7	365	356	390	397	397	407	385.2	7.5	-	-
17-May-92	+32	370	418	352	346	367	415	377.9	11.7	366	366	360	371	335	351	358.0	5.0	369	385	396	390	396	397	388.8	4.1	-	-
24-May-92	+33	364	354	347	368	357	393	363.9	6.0	379	382	346	393	384	377	376.9	6.0	356	387	426	369	305	397	373.3	15.4	-	-
31-May-92	+34	388	**	423	283	405	401	379.9	22.2	306	326	362	346	395	379	352.4	12.4	353	352	388	353	366	346	359.6	5.7	-	-
07-Jun-92	+35	348	**	393	376	402	340	371.5	10.9	364	376	339	391	390	373	372.2	7.1	394	339	450	422	337	347	381.4	17.9	0.2	0.9191

Statistics = results of Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;
**** = animal dead; the weeks when the differences between the groups were significant are printed in bold faces.**

Appendix Table 8.3.12: Total WBC counts (x109 cell/l) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
Date	WPI	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	11.8	12.3	9.5	11.8	12.2	12.3	11.65	0.40	11.5	9.8	10.5	11.4	11.0	9.5	10.62	0.31	11.2	10.6	10.5	11.3	10.8	11.2	10.93	0.13	5.4	0.0678
08-Sep-91	-4	12.4	11.8	9.8	11.7	12.5	11.9	11.68	0.36	12.2	9.5	10.4	11.2	10.8	9.8	10.65	0.37	11.0	11.0	10.7	11.5	10.8	10.9	10.98	0.10	-	-
15-Sep-91	-3	12.7	14.0	12.8	8.1	9.1	10.5	11.20	0.88	14.9	13.7	6.3	9.4	6.6	11.2	10.35	1.34	13.7	8.1	14.7	13.2	8.8	8.1	11.11	1.16	-	-
22-Sep-91	-2	13.0	15.0	12.4	7.8	8.1	10.1	11.04	1.06	15.7	14.8	5.6	8.7	7.4	12.9	10.83	1.55	13.6	9.5	14.7	6.5	9.9	9.5	10.61	1.13	-	-
29-Sep-91	-1	14.1	14.5	11.7	11.4	9.5	12.9	12.34	0.70	13.5	14.1	13.2	13.3	11.6	12.9	13.11	0.31	14.3	24.5	15.9	8.5	10.5	7.6	13.57	2.34	-	-
06-Oct-91	0	18.6	17.6	14.2	13.3	12.8	17.2	15.62	0.93	22.4	20.1	16.7	16.5	14.0	18.3	17.99	1.09	19.2	19.4	18.2	10.5	11.1	8.2	14.43	1.88	1.7	0.4208
13-Oct-91	+1	13.0	10.9	10.3	9.2	7.9	10.6	10.30	0.63	13.6	14.3	9.1	12.0	13.3	14.5	12.79	0.74	18.9	14.1	15.3	7.1	10.9	8.1	12.40	1.68	-	-
20-Oct-91	+2	28.6	16.3	17.7	16.2	15.7	20.2	19.12	1.83	27.1	25.1	16.3	19.8	20.4	19.0	21.28	1.51	19.0	25.2	24.8	10.0	18.3	12.7	18.32	2.30	-	-
27-Oct-91	+3	24.4	18.6	25.4	15.7	24.2	20.7	21.50	1.44	28.1	19.7	16.7	21.8	17.7	23.0	21.15	1.54	27.8	32.9	26.5	13.8	18.7	14.8	22.40	2.89	-	-
03-Nov-91	+4	26.6	19.2	22.7	15.1	15.7	22.0	20.22	1.65	29.3	18.3	14.1	20.3	24.6	24.5	21.85	2.01	27.3	28.7	24.0	15.4	13.4	11.8	20.09	2.77	-	-
10-Nov-91	+5	29.8	27.2	21.6	13.1	14.4	18.5	20.77	2.51	24.0	14.6	16.2	18.4	14.7	19.5	17.91	1.34	20.4	16.6	21.3	12.9	14.6	17.9	17.27	1.21	0.1	0.9445
17-Nov-91	+6	20.7	17.5	21.2	23.1	14.7	20.2	19.57	1.12	22.4	23.8	12.1	27.2	13.9	19.3	19.80	2.19	20.2	25.5	26.3	9.9	16.4	11.7	18.34	2.56	-	-
24-Nov-91	+7	25.4	18.4	23.2	16.2	11.4	18.6	18.87	1.86	28.9	24.6	18.1	21.2	16.4	21.4	21.77	1.68	22.5	25.1	23.2	8.5	12.9	10.2	17.09	2.73	-	-
01-Dec-91	+8	22.1	13.8	22.0	16.4	16.9	11.8	17.18	1.57	22.5	22.4	15.7	21.4	13.2	21.4	19.44	1.48	20.8	26.2	24.3	12.7	13.3	11.8	18.19	2.38	-	-
08-Dec-91	+9	22.9	12.7	18.7	15.1	15.6	21.2	17.71	1.46	21.1	25.2	15.2	17.2	14.0	20.2	18.83	1.55	17.3	28.4	26.3	11.6	14.0	13.6	18.53	2.65	-	-
15-Dec-91	+10	27.4	19.9	18.6	21.7	19.4	18.6	20.93	1.26	19.3	17.4	15.1	15.9	13.9	22.3	17.31	1.15	24.2	28.7	22.7	12.3	13.1	12.8	18.97	2.65	0.4	0.8340
22-Dec-91	+11	29.9	17.1	22.2	18.2	23.0	24.8	22.54	1.73	20.7	22.2	16.5	15.1	13.2	17.5	17.54	1.26	21.9	22.1	24.3	11.6	11.3	14.6	17.64	2.16	-	-
29-Dec-91	+12	20.7	14.9	19.5	21.2	27.5	18.4	20.36	1.54	18.9	19.0	19.7	14.3	16.2	22.7	18.46	1.08	26.5	23.4	20.7	12.6	14.6	12.5	18.39	2.23	-	-
05-Jan-92	+13	28.2	18.2	28.8	21.0	25.5	21.4	23.84	1.60	23.5	22.5	17.8	15.2	15.3	27.4	20.29	1.85	22.6	26.3	22.3	13.7	19.6	14.3	19.81	1.85	-	-
12-Jan-92	+14	26.9	24.4	22.6	20.8	20.4	20.2	22.53	0.99	21.6	24.9	18.7	13.5	15.8	25.5	19.98	1.80	21.7	29.0	21.6	14.1	13.6	14.7	19.11	2.27	-	-
19-Jan-92	+15	26.4	23.5	21.8	16.8	20.9	10.7	20.02	2.07	22.7	23.8	20.3	18.0	10.8	22.8	19.73	1.82	22.6	29.8	21.4	11.8	16.8	15.8	19.71	2.36	0.2	0.9119
26-Jan-92	+16	26.4	17.1	14.8	25.9	21.9	15.3	20.24	1.95	18.8	21.8	14.3	13.7	13.5	19.2	16.91	1.31	25.5	26.0	22.6	11.6	14.4	13.7	18.98	2.40	-	-
02-Feb-92	+17	26.6	19.7	23.1	17.1	15.6	16.1	19.70	1.63	17.6	19.9	18.4	15.1	13.7	16.1	16.82	0.84	21.0	26.9	28.9	12.5	12.8	12.6	19.11	2.81	-	-
09-Feb-92	+18	30.6	15.9	21.3	20.8	17.5	15.1	20.20	2.12	21.7	24.3	20.4	19.0	15.7	21.7	20.48	1.08	28.1	20.4	23.7	13.1	13.8	17.5	19.42	2.18	-	-
16-Feb-92	+19	26.6	26.0	17.4	13.7	14.2	16.3	19.03	2.16	19.3	19.8	20.5	16.7	13.9	24.3	19.07	1.32	29.7	16.9	17.3	17.8	13.6	17.5	18.81	2.07	-	-
23-Feb-92	+20	25.1	27.6	24.2	12.6	12.5	13.9	19.31	2.63	15.4	19.5	14.4	17.7	11.6	21.4	16.67	1.33	26.1	13.1	16.0	15.2	12.5	15.7	16.46	1.85	0.2	0.9066
01-Mar-92	+21	24.1	28.7	21.5	11.4	14.6	12.9	18.90	2.59	15.6	22.8	16.5	16.2	9.8	19.8	16.79	1.64	28.2	14.2	16.9	15.9	15.7	17.1	18.01	1.89	-	-
08-Mar-92	+22	24.1	24.3	22.3	12.3	13.2	15.0	18.52	2.10	16.8	20.2	16.2	15.5	13.9	17.3	16.66	0.79	29.1	13.3	14.0	16.7	19.2	13.4	17.62	2.27	-	-
15-Mar-92	+23	26.4	26.1	21.4	12.2	12.6	13.0	18.60	2.55	12.5	16.8	18.1	15.7	9.8	22.4	15.87	1.64	25.1	14.8	14.4	14.6	12.3	17.3	16.42	1.70	-	-
22-Mar-92	+24	19.2	24.0	21.3	14.0	13.9	15.8	18.04	1.54	15.4	19.6	18.2	13.6	10.2	19.6	16.11	1.39	28.3	13.4	17.1	12.4	18.4	17.0	17.76	2.12	-	-
29-Mar-92	+25	31.7	23.6	21.5	13.8	13.1	12.9	19.45	2.82	16.7	19.2	20.0	15.0	11.0	17.7	16.59	1.22	24.1	13.6	18.0	14.5	11.2	15.2	16.09	1.68	0.3	0.8640
05-Apr-92	+26	25.2	26.0	26.4	11.9	13.7	17.4	20.10	2.45	14.3	20.1	16.7	13.4	8.5	15.9	14.84	1.44	24.9	15.1	13.2	14.7	14.5	13.0	15.91	1.68	-	-
12-Apr-92	+27	25.3	23.7	21.5	11.1	12.9	12.0	17.77	2.40	13.1	15.2	16.6	14.1	8.6	17.2	14.14	1.16	18.9	12.1	15.2	13.1	10.6	16.3	14.37	1.13	-	-
19-Apr-92	+28	24.6	20.9	22.4	13.1	15.9	12.0	18.14	1.94	15.3	24.6	16.6	13.1	10.2	18.1	16.31	1.84	17.9	13.4	13.1	15.6	15.1	17.4	15.41	0.74	-	-
26-Apr-92	+29	23.2	20.9	20.6	13.4	14.4	12.8	17.54	1.69	12.6	18.8	12.1	13.1	9.6	22.2	14.73	1.77	19.5	12.0	12.0	13.5	10.4	14.3	13.63	1.18	-	-
03-May-92	+30	17.3	21.7	20.5	11.6	14.2	13.7	16.49	1.50	12.2	21.2	15.3	12.1	10.5	11.3	13.76	1.49	19.0	14.4	11.3	19.0	14.8	13.3	15.30	1.16	1.8	0.4038
10-May-92	+31	22.5	20.0	19.7	12.8	14.4	11.3	16.79	1.69	11.6	16.9	13.3	11.9	9.0	16.7	13.23	1.16	20.6	14.1	11.7	12.8	7.6	14.6	13.57	1.59	-	-
17-May-92	+32	23.1	20.0	18.1	12.0	12.3	13.5	16.51	1.71	11.3	18.0	12.7	12.7	9.4	20.2	14.06	1.55	19.6	14.0	11.7	14.0	9.6	16.5	14.26	1.31	-	-
24-May-92	+33	26.2	25.6	20.0	18.8	13.9	18.1	20.43	1.76	16.5	15.9	17.0	13.8	10.4	20.9	15.76	1.31	20.8	16.8	11.5	14.3	10.4	19.1	15.49	1.55	-	-
31-May-92	+34	17.6	**	16.2	16.2	19.5	14.2	16.74	0.78	11.3	16.5	13.8	13.8	9.4	18.0	13.81	1.18	28.2	17.5	17.4	12.4	10.2	13.1	16.47	2.39	-	-
07-Jun-92	+35	24.8	**	23.4	14.9	18.2	16.0	19.46	1.78	13.5	18.9	15.6	12.8	12.6	17.3	15.13	0.97	23.3	22.4	16.0	13.3	13.1	12.4	16.74	1.82	0.4	0.8161

Statistics = results of Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;
**** = animal dead; the week when the differences between the groups were significant are printed in bold faces.**

Appendix Table 8.3.13: Eosinophil counts (x10:9 cell/l) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
Date	WPI	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	0.28	0.24	0.23	0.23	0.25	0.28	0.25	0.01	0.24	0.21	0.24	0.27	0.26	0.24	0.24	0.01	0.27	0.23	0.26	0.27	0.27	0.28	0.26	0.01	2.1	0.3435
08-Sep-91	-4	0.25	0.37	0.42	0.18	0.19	0.19	0.27	0.04	0.32	0.28	0.22	0.28	0.28	0.18	0.26	0.02	0.38	0.38	0.24	0.35	0.38	0.16	0.32	0.03	-	-
15-Sep-91	-3	0.22	0.35	0.38	0.14	0.07	0.25	0.24	0.04	0.35	0.19	0.25	0.30	0.16	0.16	0.23	0.03	0.35	0.40	0.28	0.39	0.39	0.26	0.35	0.02	-	-
22-Sep-91	-2	0.14	0.28	0.47	0.09	0.33	0.13	0.24	0.06	0.28	0.29	0.27	0.17	0.36	0.08	0.24	0.04	0.45	0.39	0.20	0.34	0.33	0.07	0.30	0.05	-	-
29-Sep-91	-1	0.11	0.23	0.45	0.04	0.13	0.18	0.19	0.05	0.30	0.21	0.27	0.12	0.10	0.23	0.20	0.03	0.30	0.42	0.22	0.12	0.30	0.11	0.24	0.05	-	-
06-Oct-91	0	0.28	0.35	0.47	0.12	0.12	0.20	0.26	0.05	0.08	0.27	0.19	0.28	0.33	0.12	0.21	0.04	0.14	0.42	0.23	0.21	0.18	0.20	0.23	0.04	0.3	0.8493
13-Oct-91	+1	0.12	0.43	0.73	0.43	0.58	0.12	0.40	0.09	0.31	0.18	0.28	0.28	0.21	0.30	0.26	0.02	0.15	0.33	0.15	0.27	0.13	0.26	0.22	0.03	2.0	0.3642
20-Oct-91	+2	0.28	0.60	1.09	0.54	1.17	0.25	0.66	0.15	0.34	0.37	0.38	0.33	0.35	0.32	0.35	0.01	0.12	0.37	0.13	0.28	0.18	0.12	0.20	0.04	7.4	0.0248
27-Oct-91	+3	0.23	0.54	3.03	0.95	3.81	0.19	1.46	0.58	0.72	0.49	0.23	0.37	0.10	0.22	0.36	0.08	0.19	0.35	0.14	0.22	0.11	0.08	0.18	0.04	6.8	0.0335
03-Nov-91	+4	0.24	0.53	2.10	0.88	2.28	0.21	1.04	0.35	0.80	0.64	0.36	0.42	0.43	0.18	0.47	0.08	0.16	0.37	0.14	0.14	0.08	0.07	0.16	0.04	9.4	0.0090
10-Nov-91	+5	0.37	0.22	2.33	0.94	2.81	0.17	1.14	0.43	0.42	0.26	0.68	0.91	0.30	0.04	0.43	0.12	0.11	0.35	0.18	0.15	0.09	0.08	0.16	0.04	6.1	0.0464
17-Nov-91	+6	0.23	0.20	0.60	0.40	2.28	0.15	0.64	0.31	0.19	0.17	0.14	2.90	0.11	0.20	0.62	0.42	0.08	0.28	0.21	0.12	0.08	0.08	0.14	0.03	-	-
24-Nov-91	+7	0.13	0.24	0.79	0.28	1.37	0.22	0.51	0.18	0.08	0.18	0.37	1.13	0.27	0.09	0.35	0.15	0.11	0.23	0.26	0.11	0.06	0.10	0.14	0.03	-	-
01-Dec-91	+8	0.09	0.58	0.73	0.14	2.03	0.38	0.66	0.27	0.03	0.16	0.26	1.41	0.08	0.11	0.34	0.20	0.13	0.26	0.20	0.15	0.06	0.17	0.16	0.02	-	-
08-Dec-91	+9	0.05	0.34	0.72	0.28	1.29	0.29	0.50	0.17	0.10	0.18	0.28	0.30	0.03	0.18	0.18	0.04	0.20	0.29	0.20	0.17	0.11	0.17	0.19	0.02	-	-
15-Dec-91	+10	0.23	0.81	2.02	0.34	4.62	0.21	1.37	0.65	0.39	0.07	0.26	0.72	0.35	0.27	0.34	0.08	0.14	0.26	0.14	0.20	0.18	0.12	0.17	0.02	7.3	0.0265
22-Dec-91	+11	0.18	0.39	2.50	2.15	8.95	0.85	2.50	1.23	0.28	0.19	0.24	0.48	0.21	0.16	0.26	0.04	0.16	0.26	0.12	0.21	0.10	0.24	0.18	0.02	-	-
29-Dec-91	+12	0.30	1.10	5.29	7.15	7.64	1.44	3.82	1.22	1.57	0.48	0.45	0.70	0.20	0.20	0.60	0.19	0.16	0.28	0.19	0.25	0.19	0.24	0.22	0.02	-	-
05-Jan-92	+13	0.15	1.73	6.25	8.94	8.56	4.02	4.94	1.34	2.54	0.28	0.92	0.42	0.46	0.23	0.81	0.33	0.18	0.21	0.15	0.28	0.18	0.20	0.20	0.02	-	-
12-Jan-92	+14	0.62	0.51	5.47	8.30	8.02	2.14	4.18	1.33	3.66	0.42	1.62	1.00	0.38	0.19	1.21	0.49	0.22	0.17	0.21	0.23	0.14	0.23	0.20	0.01	-	-
19-Jan-92	+15	0.26	0.28	2.79	5.45	7.13	0.72	2.77	1.09	0.83	0.30	0.55	0.30	0.23	0.17	0.40	0.09	0.21	0.18	0.28	0.25	0.17	0.24	0.22	0.02	7.8	0.0204
26-Jan-92	+16	0.17	0.31	0.84	4.69	3.31	0.31	1.61	0.72	0.27	0.07	0.42	0.36	0.54	0.18	0.31	0.06	0.21	0.28	0.21	0.25	0.25	0.23	0.24	0.01	-	-
02-Feb-92	+17	0.17	0.61	7.13	4.92	8.14	0.35	3.55	1.35	0.39	0.17	0.32	0.34	2.98	0.17	0.73	0.41	0.39	0.29	0.29	0.26	0.21	0.23	0.28	0.02	-	-
09-Feb-92	+18	0.09	0.26	4.31	4.13	4.22	0.19	2.20	0.83	0.08	0.14	0.17	0.77	3.28	0.23	0.78	0.47	0.31	0.25	0.14	0.32	0.23	0.18	0.24	0.03	-	-
16-Feb-92	+19	0.33	0.45	1.34	2.07	2.23	0.25	1.11	0.33	0.63	0.15	0.18	3.38	0.64	0.21	0.87	0.47	0.30	0.28	0.17	0.33	0.28	0.33	0.28	0.02	-	-
23-Feb-92	+20	0.38	0.42	2.41	0.64	2.39	0.28	1.09	0.38	0.17	0.13	0.14	2.84	0.09	0.16	0.59	0.41	0.31	0.23	0.23	0.27	0.33	0.21	0.26	0.02	8.1	0.0178
01-Mar-92	+21	0.38	0.40	2.64	0.76	4.66	0.42	1.54	0.66	0.12	0.30	0.30	0.32	0.15	0.13	0.22	0.04	0.38	0.25	0.14	0.19	0.19	0.19	0.22	0.03	11.2	0.0037
08-Mar-92	+22	0.56	0.45	1.58	1.88	1.11	0.38	0.99	0.24	0.14	0.07	0.12	0.36	0.12	0.22	0.17	0.04	0.25	0.29	0.30	0.20	0.12	0.30	0.24	0.03	12.2	0.0023
15-Mar-92	+23	0.19	0.66	2.97	1.42	3.93	0.45	1.60	0.57	0.07	0.32	0.13	0.30	0.21	0.02	0.17	0.05	0.32	0.21	0.14	0.15	0.19	0.37	0.23	0.04	8.1	0.0173
22-Mar-92	+24	0.27	0.26	2.21	0.97	6.53	0.33	1.76	0.91	0.19	0.12	0.22	0.12	0.17	0.13	0.16	0.02	0.31	0.21	0.29	0.26	0.13	0.19	0.23	0.03	11.1	0.0038
29-Mar-92	+25	0.15	0.23	1.29	3.04	3.04	0.17	1.32	0.52	0.13	0.17	0.14	0.18	0.34	0.43	0.23	0.05	0.35	0.15	0.13	0.26	0.14	0.34	0.23	0.04	2.6	0.2789
05-Apr-92	+26	0.05	0.51	0.35	1.43	1.64	0.03	0.67	0.26	0.01	0.03	0.01	0.02	0.03	0.05	0.03	0.01	0.31	0.08	0.02	0.11	0.02	0.17	0.12	0.04	-	-
12-Apr-92	+27	0.18	0.71	0.46	0.41	2.49	0.45	0.78	0.32	0.06	0.13	0.32	0.41	0.08	0.13	0.19	0.05	0.30	0.21	0.10	0.23	0.27	0.24	0.22	0.03	-	-
19-Apr-92	+28	0.41	0.49	0.87	2.60	2.25	0.25	1.15	0.38	0.07	0.26	0.32	0.34	0.22	0.33	0.25	0.04	0.37	0.37	0.29	0.13	0.26	0.21	0.27	0.04	-	-
26-Apr-92	+29	0.60	0.34	0.33	2.23	1.57	0.26	0.89	0.31	0.06	0.32	0.22	0.77	0.24	0.11	0.29	0.09	0.24	0.34	0.13	0.28	0.23	0.24	0.24	0.03	-	-
03-May-92	+30	0.34	0.27	0.15	4.95	2.55	0.13	1.40	0.74	0.12	0.10	3.89	1.13	0.08	0.13	0.91	0.57	0.24	0.26	0.29	0.27	0.26	0.28	0.27	0.01	2.1	0.3474
10-May-92	+31	0.08	0.13	0.15	1.69	2.35	0.23	0.77	0.37	0.06	1.27	0.10	0.48	0.27	0.17	0.39	0.17	0.27	0.23	0.28	0.30	0.25	0.27	0.27	0.01	-	-
17-May-92	+32	0.05	0.10	1.13	1.47	1.60	0.23	0.76	0.27	0.06	0.06	0.07	0.28	0.10	0.09	0.11	0.03	0.10	0.29	0.28	0.25	0.16	0.12	0.20	0.03	-	-
24-May-92	+33	0.16	0.13	0.92	1.96	0.11	0.06	0.56	0.28	0.13	0.10	0.32	0.17	0.11	0.19	0.17	0.03	0.27	0.32	0.33	0.28	0.24	0.11	0.26	0.03	-	-
31-May-92	+34	0.07	**	1.69	1.14	1.47	0.26	0.92	0.29	0.06	0.07	0.11	0.19	0.20	0.18	0.13	0.02	0.28	0.32	0.16	0.24	0.14	0.12	0.21	0.03	-	-
07-Jun-92	+35	0.06	**	0.88	1.09	1.10	0.09	0.64	0.21	0.04	0.18	0.18	0.22	0.19	0.07	0.15	0.03	0.29	0.28	0.20	0.21	0.19	0.13	0.22	0.02	2.8	0.2479

Statistics = results of Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;
**** = animal dead; the week when the differences between the groups were significant are printed in bold faces.**

Appendix Table 8.3.14: Serum Total Protein Concentrations (g/l) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
Date	WPI	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	59.4	67.2	67.2	59.4	71.2	64.1	64.75	1.76	61.0	61.6	64.1	64.3	63.4	72.6	64.50	1.56	63.5	71.4	59.0	65.4	47.6	65.2	62.02	3.02	0.2	0.8985
08-Sep-91	-4	69.8	66.2	70.4	58.2	74.4	72.3	68.55	2.15	56.9	60.1	59.6	62.3	65.3	60.3	60.75	1.05	58.3	69.3	69.0	68.3	53.5	68.3	64.45	2.54	-	-
15-Sep-91	-3	56.9	60.1	59.6	62.3	65.3	60.3	60.75	1.05	65.3	64.1	58.5	65.8	68.7	65.1	64.58	1.25	54.5	60.6	65.8	66.5	68.1	59.0	62.42	1.96	-	-
22-Sep-91	-2	61.0	61.6	64.1	64.3	63.4	72.6	64.50	1.56	69.8	66.2	70.4	58.2	74.4	72.3	68.55	2.15	59.4	67.2	67.2	59.4	71.2	64.1	64.75	1.76	-	-
29-Sep-91	-1	70.7	58.1	63.6	58.3	62.8	68.4	63.65	1.92	53.8	61.7	66.5	64.8	70.6	63.7	63.52	2.10	67.5	60.8	63.6	61.2	62.8	57.2	62.18	1.27	-	-
06-Oct-91	0	62.4	62.4	74.1	66.3	45.0	75.1	64.22	4.07	76.0	80.0	66.3	69.2	61.4	66.0	69.82	2.58	62.4	67.7	63.9	68.2	68.2	58.5	64.82	1.47	1.4	0.5001
13-Oct-91	+1	66.0	72.8	77.0	63.5	67.2	78.1	70.77	2.27	73.5	64.0	73.3	65.2	79.3	69.3	70.77	2.14	57.9	73.0	67.5	68.2	67.7	68.8	67.18	1.86	-	-
20-Oct-91	+2	65.8	70.8	75.4	66.8	63.3	79.3	70.23	2.29	71.4	67.7	76.0	65.0	82.0	70.9	72.17	2.27	63.6	86.6	70.1	71.4	73.1	67.1	71.98	2.95	-	-
27-Oct-91	+3	59.2	60.6	64.3	58.3	61.4	68.4	62.03	1.40	64.1	61.8	64.3	57.6	71.1	66.2	64.18	1.67	56.5	72.9	63.5	72.5	69.1	57.1	65.27	2.75	-	-
03-Nov-91	+4	61.4	69.1	74.8	62.3	66.0	68.4	67.00	1.84	70.0	61.7	67.9	66.8	83.0	72.4	70.29	2.68	58.3	69.3	69.0	68.3	53.5	68.3	64.45	2.54	-	-
10-Nov-91	+5	52.6	57.7	60.8	60.1	64.7	67.4	60.47	1.91	63.7	56.9	66.6	60.0	65.6	69.2	63.67	1.68	57.5	69.9	68.1	69.6	58.6	66.7	65.07	2.07	2.1	0.3429
17-Nov-91	+6	70.4	65.7	71.6	65.4	69.7	67.7	68.42	0.95	76.9	71.2	67.1	69.7	68.1	68.5	70.25	1.32	60.5	74.2	72.1	70.4	64.4	65.4	67.83	1.95	-	-
24-Nov-91	+7	60.1	69.4	68.9	64.0	64.3	70.5	66.20	1.51	66.8	61.5	59.6	58.6	69.4	64.5	63.40	1.58	58.4	58.8	58.0	62.2	53.9	60.0	58.55	1.02	-	-
01-Dec-91	+8	60.1	61.3	68.8	64.0	64.0	68.7	64.48	1.36	66.6	64.5	66.2	63.7	74.1	78.3	68.90	2.20	60.2	70.5	68.0	61.9	58.3	68.7	64.60	1.90	-	-
08-Dec-91	+9	65.0	69.7	68.4	68.4	75.1	79.4	71.00	1.96	73.4	73.4	65.4	63.4	80.8	81.0	72.90	2.76	63.0	73.2	66.2	66.9	60.7	72.5	67.08	1.86	-	-
15-Dec-91	+10	68.7	81.2	74.8	77.6	77.8	78.5	76.43	1.60	76.7	72.5	70.8	69.9	81.2	76.3	74.57	1.60	71.2	83.6	76.3	69.2	64.6	77.2	73.68	2.51	1.2	0.5493
22-Dec-91	+11	60.0	68.9	69.5	72.3	75.8	70.8	69.55	1.97	67.6	61.1	66.7	60.7	71.7	73.1	66.82	1.93	59.6	68.6	60.0	70.3	49.0	69.1	62.77	3.06	-	-
29-Dec-91	+12	66.7	63.7	65.1	64.9	78.4	83.0	70.30	3.07	73.0	68.1	71.1	63.1	74.1	78.0	71.23	1.92	65.4	74.6	69.2	68.3	56.4	73.2	67.85	2.43	-	-
05-Jan-92	+13	68.9	73.8	82.3	78.8	87.4	85.9	79.52	2.67	76.8	73.1	69.9	71.5	77.4	78.4	74.52	1.30	65.6	74.4	68.3	68.7	54.9	71.9	67.30	2.53	-	-
12-Jan-92	+14	63.4	64.9	72.9	69.7	73.8	80.7	70.90	2.37	76.4	63.6	63.6	62.7	67.0	67.1	66.73	1.90	59.9	74.8	64.9	64.7	66.6	61.4	65.38	1.95	-	-
19-Jan-92	+15	57.1	61.6	71.9	63.9	72.8	51.5	63.13	3.10	73.8	63.9	66.3	60.7	66.3	68.2	66.53	1.64	63.5	71.4	59.0	65.4	57.6	65.2	63.68	1.85	1.7	0.4358
26-Jan-92	+16	64.1	65.5	81.7	76.5	76.7	79.2	73.95	2.74	75.3	74.6	67.6	72.4	71.1	72.2	72.20	1.02	64.7	75.9	69.3	69.2	58.6	66.6	67.38	2.14	-	-
02-Feb-92	+17	62.1	65.5	68.3	72.7	68.0	81.9	69.74	2.57	78.5	79.6	71.3	64.9	75.3	77.6	74.54	2.06	69.5	81.5	71.5	77.7	64.4	74.2	73.13	2.27	-	-
09-Feb-92	+18	68.2	61.4	70.5	67.2	64.3	73.4	67.49	1.60	70.9	70.9	68.2	64.1	76.7	68.6	69.91	1.55	64.1	78.8	70.9	68.6	59.5	69.3	68.53	2.42	-	-
16-Feb-92	+19	63.2	63.4	70.6	67.0	57.6	74.7	66.10	2.25	74.9	69.6	61.4	61.9	74.7	74.0	69.43	2.36	66.1	78.7	67.0	67.2	62.7	72.5	69.02	2.12	-	-
23-Feb-92	+20	61.7	67.0	69.2	61.1	56.4	76.9	65.39	2.69	75.3	74.3	68.2	67.0	71.7	72.1	71.45	1.22	70.8	82.6	67.2	69.4	63.9	68.0	70.30	2.40	3.5	0.1771
01-Mar-92	+21	60.0	60.9	59.2	62.4	54.5	77.8	62.46	2.96	66.4	69.2	62.0	59.0	71.1	67.3	65.81	1.69	70.9	80.5	61.7	69.6	63.5	69.1	69.23	2.47	-	-
08-Mar-92	+22	65.5	61.2	58.4	56.3	56.5	71.5	61.60	2.23	66.3	69.7	64.6	63.5	71.7	70.6	67.73	1.27	64.0	76.8	66.3	70.6	60.5	72.9	68.51	2.25	-	-
15-Mar-92	+23	63.6	65.5	63.4	60.1	51.7	73.4	62.97	2.65	72.3	70.9	67.9	65.5	71.5	71.5	69.94	0.99	66.1	78.8	70.0	74.0	59.6	71.1	69.94	2.47	-	-
22-Mar-92	+24	62.7	64.8	57.9	58.1	45.9	75.8	60.89	3.66	63.2	64.5	71.9	66.0	78.7	74.3	69.76	2.30	69.3	77.4	72.6	74.3	65.1	71.3	71.67	1.57	-	-
29-Mar-92	+25	62.8	60.5	57.1	61.3	54.0	75.4	61.85	2.74	66.9	65.9	64.0	60.3	72.7	72.9	67.12	1.83	66.2	76.0	70.8	65.9	63.8	67.7	68.39	1.63	4.5	0.1061
05-Apr-92	+26	58.7	55.1	54.6	56.5	45.1	64.6	55.77	2.37	56.2	63.8	60.5	65.0	63.4	68.9	62.97	1.60	56.5	52.0	60.3	61.7	59.3	63.9	58.95	1.57	-	-
12-Apr-92	+27	58.3	61.1	60.2	57.2	43.9	75.3	59.35	3.74	63.6	71.0	63.4	63.9	67.3	82.8	68.66	2.81	67.1	72.9	60.6	70.1	60.7	66.9	66.39	1.85	-	-
19-Apr-92	+28	60.6	64.5	59.5	56.1	46.0	73.4	60.01	3.38	68.4	67.0	68.6	67.0	67.9	71.4	68.39	0.61	64.5	73.9	63.6	70.6	62.4	70.0	67.50	1.73	-	-
26-Apr-92	+29	64.6	57.8	69.3	53.1	49.8	78.7	62.22	4.02	63.0	68.7	77.6	68.1	66.1	75.0	69.75	2.06	65.7	79.6	69.8	72.8	66.3	72.4	71.11	1.91	5.4	0.0656
03-May-92	+30	53.2	52.5	50.5	48.9	35.8	58.5	49.90	2.85	54.1	57.1	62.0	54.6	64.5	66.0	59.72	1.91	62.0	72.2	63.8	70.1	76.2	68.4	68.77	1.98	12.2	0.0022
10-May-92	+31	57.8	56.0	54.4	54.1	39.9	65.9	54.68	3.16	57.2	61.7	64.4	65.0	62.6	73.7	64.12	2.03	64.5	70.9	61.6	68.7	66.9	60.8	65.56	1.48	6.6	0.0363
17-May-92	+32	55.4	58.5	55.4	54.0	39.3	62.1	54.11	2.91	58.8	60.5	59.0	56.2	67.9	69.2	61.92	1.99	66.0	65.5	63.1	66.1	64.5	60.5	64.28	0.80	8.6	0.0138
24-May-92	+33	51.1	54.7	47.8	49.5	34.7	63.6	50.23	3.52	53.9	57.5	59.4	61.4	62.4	61.8	59.42	1.21	65.1	69.0	66.9	62.1	61.1	64.7	64.81	1.09	9.6	0.0083
31-May-92	+34	52.3	**	53.2	49.7	41.7	62.8	51.95	3.04	55.8	62.4	59.9	55.1	65.7	68.0	61.15	1.94	60.0	68.1	63.7	65.1	65.3	71.8	65.67	1.49	8.4	0.0151
07-Jun-92	+35	56.1	**	56.0	54.4	43.7	61.3	54.30	2.60	59.8	57.8	60.8	55.1	63.0	61.7	59.70	1.07	61.9	61.0	67.5	63.1	66.4	65.5	64.24	0.98	9.9	0.0072

Statistics = results of Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;
** = animal dead; the weeks when differences between the groups were significant are printed in bold faces.

Appendix Table 8.3.15: Serum Albumin Concentrations (g/l) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
Date	WPI	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	34.7	32.5	31.1	30.2	34.7	32.5	32.62	0.68	28.5	31.7	33.8	34.0	30.7	32.3	31.83	0.77	28.5	37.0	37.0	34.5	28.5	37.0	33.75	1.56	1.6	0.4557
08-Sep-91	-4	29.8	31.2	32.0	34.2	31.3	33.4	31.98	0.60	32.9	34.8	32.9	34.8	32.9	34.8	33.85	0.39	34.0	32.0	32.6	30.4	34.0	32.0	32.50	0.51	-	-
15-Sep-91	-3	28.5	31.7	33.8	34.0	30.7	32.3	31.83	0.77	35.4	35.6	35.4	35.6	35.4	35.6	35.50	0.04	34.7	32.5	31.1	30.2	34.7	32.5	32.62	0.68	-	-
22-Sep-91	-2	29.4	32.3	33.8	33.4	29.8	34.7	32.23	0.81	33.7	34.4	33.7	34.4	33.7	34.4	34.05	0.14	33.2	30.3	33.8	31.7	33.2	30.3	32.08	0.58	-	-
29-Sep-91	-1	29.8	30.7	31.4	32.4	31.4	32.4	31.35	0.37	33.2	34.2	33.2	34.2	33.2	34.2	33.70	0.20	31.9	30.9	34.0	30.7	31.9	30.9	31.72	0.46	-	-
06-Oct-91	0	31.8	33.3	32.3	32.4	33.3	34.4	32.92	0.35	34.2	30.2	34.2	30.2	34.2	30.2	32.20	0.82	33.3	38.5	31.2	33.6	33.3	38.5	34.73	1.13	1.6	0.4523
13-Oct-91	+1	32.8	33.1	33.3	35.4	33.8	32.6	33.50	0.38	35.0	35.5	35.0	35.5	35.0	35.5	35.25	0.10	34.6	35.1	33.1	36.2	34.6	35.1	34.78	0.38	-	-
20-Oct-91	+2	33.4	32.5	31.2	36.5	34.7	35.3	33.93	0.72	34.5	32.2	34.5	32.2	34.5	32.2	33.35	0.47	36.4	34.5	35.8	36.8	36.4	34.5	35.73	0.38	-	-
27-Oct-91	+3	31.9	32.2	35.0	33.0	33.7	33.8	33.27	0.43	35.4	33.9	35.4	33.9	35.4	33.9	34.65	0.31	35.1	30.9	35.4	36.6	35.1	30.9	34.00	0.92	-	-
03-Nov-91	+4	29.5	41.0	40.5	31.3	31.1	28.8	33.70	2.07	27.3	33.0	32.7	33.0	37.3	33.0	32.72	1.19	28.5	37.0	37.0	34.5	28.5	37.0	33.75	1.56	-	-
10-Nov-91	+5	31.1	33.6	35.8	37.1	37.5	35.4	35.08	0.89	37.3	32.7	37.3	32.7	37.3	32.7	35.00	0.94	34.5	30.4	32.0	31.3	32.5	34.4	32.52	0.62	4.7	0.0962
17-Nov-91	+6	30.4	31.6	33.3	31.9	34.3	33.7	32.53	0.55	34.3	32.7	34.3	32.7	34.3	32.7	33.50	0.33	29.2	32.9	36.0	36.0	32.2	29.2	32.59	1.14	-	-
24-Nov-91	+7	31.3	32.2	35.2	31.0	34.0	37.4	33.52	0.93	34.0	32.2	38.0	35.2	38.0	35.2	35.43	0.85	30.7	30.4	33.6	32.1	30.7	30.4	31.32	0.48	-	-
01-Dec-91	+8	33.7	33.6	30.0	32.1	34.7	36.8	33.48	0.86	34.9	31.5	34.9	31.5	34.9	36.5	34.03	0.77	31.8	32.1	32.3	31.1	31.8	34.1	32.20	0.38	-	-
08-Dec-91	+9	32.3	32.1	32.3	30.2	36.4	38.5	33.63	1.17	33.5	34.6	33.5	34.6	38.5	34.6	34.88	0.69	37.5	30.6	35.8	37.0	37.5	30.6	34.83	1.24	-	-
15-Dec-91	+10	30.6	35.7	35.4	36.8	34.2	34.5	34.53	0.80	32.9	37.8	32.9	37.8	32.9	37.8	35.35	1.00	34.9	35.1	34.3	34.6	34.9	35.1	34.82	0.12	0.1	0.9595
22-Dec-91	+11	36.4	33.4	32.4	30.6	36.3	30.8	33.32	0.96	33.8	35.6	33.8	35.6	33.8	35.6	34.70	0.37	33.5	32.7	33.0	39.8	33.5	32.7	34.20	1.03	-	-
29-Dec-91	+12	34.6	39.8	36.5	38.6	33.1	39.0	36.93	0.99	38.4	35.3	38.4	35.3	38.4	35.3	36.85	0.63	38.2	34.1	36.1	34.4	38.2	34.1	35.85	0.73	2.3	0.3141
05-Jan-92	+13	31.2	36.5	36.3	35.5	26.8	35.7	33.67	1.45	37.1	33.5	37.1	33.5	37.1	33.5	35.30	0.73	39.1	34.6	35.6	35.2	39.1	34.6	36.37	0.80	1.0	0.6196
12-Jan-92	+14	32.8	33.1	30.5	31.4	26.4	34.9	31.52	1.09	35.5	34.2	35.5	34.2	35.5	34.2	34.85	0.27	36.3	36.2	37.7	35.0	36.3	36.2	36.28	0.32	12.9	0.0016
19-Jan-92	+15	31.2	35.2	34.2	31.9	24.5	35.1	32.02	1.51	35.8	36.0	35.8	36.0	35.8	36.0	35.90	0.04	38.0	36.8	36.8	36.4	38.0	36.8	37.13	0.26	15.4	0.0005
26-Jan-92	+16	36.5	34.4	34.4	27.7	21.9	34.5	31.57	2.09	36.6	38.0	36.6	38.0	36.6	38.0	37.30	0.29	37.6	35.4	38.2	37.6	37.6	35.4	36.97	0.46	-	-
02-Feb-92	+17	33.7	33.8	33.3	32.9	21.4	36.6	31.96	1.99	37.5	36.3	37.5	36.3	37.5	36.3	36.87	0.24	37.9	36.8	39.9	39.4	37.9	36.8	38.13	0.48	-	-
09-Feb-92	+18	31.4	30.0	33.6	31.6	18.0	32.7	29.57	2.16	30.7	35.2	30.7	35.2	30.7	35.2	32.91	0.92	34.8	32.4	41.1	30.1	34.8	32.4	34.27	1.41	-	-
16-Feb-92	+19	33.9	35.4	33.8	36.3	27.6	37.0	34.00	1.27	38.5	37.0	38.5	37.0	38.5	37.0	37.75	0.31	37.5	36.8	40.6	40.3	37.5	36.8	38.26	0.64	-	-
23-Feb-92	+20	33.4	34.2	32.6	30.8	18.4	31.1	30.10	2.20	34.8	35.0	34.8	35.0	34.8	35.0	34.88	0.05	36.8	33.4	37.1	36.9	36.8	33.4	35.72	0.68	9.9	0.0070
01-Mar-92	+21	36.0	35.4	33.9	32.7	21.9	34.8	32.44	1.97	37.9	33.8	37.9	33.8	37.9	33.8	35.84	0.83	40.7	33.8	38.2	37.3	40.7	33.8	37.42	1.15	-	-
08-Mar-92	+22	31.8	32.3	29.2	32.5	18.7	35.2	29.97	2.18	35.7	33.9	35.7	33.9	35.7	33.9	34.81	0.36	37.8	32.4	37.6	38.9	37.8	32.4	36.16	1.09	-	-
15-Mar-92	+23	31.6	30.8	24.4	29.3	19.0	31.8	27.83	1.90	31.4	38.0	31.4	38.0	31.4	38.0	34.72	1.35	33.2	33.4	36.8	39.1	33.2	33.4	34.84	0.93	-	-
22-Mar-92	+24	32.3	30.9	25.9	28.4	18.1	32.9	28.08	2.07	32.4	33.3	32.4	33.3	32.4	33.3	32.85	0.18	37.9	34.8	37.8	35.1	37.9	34.3	36.28	0.64	-	-
29-Mar-92	+25	33.9	35.1	29.3	30.0	18.1	35.2	30.28	2.42	35.1	34.1	35.1	34.1	35.1	34.1	34.60	0.22	35.2	35.0	37.6	36.7	35.2	35.0	35.79	0.41	6.4	0.0417
05-Apr-92	+26	37.2	34.4	31.0	33.7	19.7	36.7	32.12	2.42	36.3	36.4	36.3	36.4	36.3	36.4	36.35	0.02	35.4	36.4	39.2	41.0	35.4	36.4	37.30	0.85	-	-
12-Apr-92	+27	31.0	31.7	29.2	26.5	16.4	32.5	27.89	2.24	33.2	29.8	33.2	29.8	33.2	29.8	31.48	0.69	34.9	31.8	33.4	32.9	34.9	31.8	33.27	0.53	-	-
19-Apr-92	+28	34.1	32.6	28.9	28.3	18.3	34.2	29.40	2.24	32.4	33.3	32.4	33.3	32.4	33.3	32.85	0.18	34.2	32.5	34.6	35.5	34.2	32.5	33.92	0.45	-	-
26-Apr-92	+29	30.5	30.6	29.9	26.1	17.4	33.1	27.94	2.09	33.5	30.0	33.5	30.0	33.5	30.0	31.71	0.72	33.9	33.0	33.1	33.9	33.9	33.0	33.45	0.18	-	-
03-May-92	+30	32.7	30.8	30.8	26.4	16.9	31.1	28.11	2.19	33.7	31.6	33.7	31.6	33.7	31.6	32.64	0.43	31.4	32.9	33.5	36.9	31.4	32.9	33.15	0.75	8.6	0.0136
10-May-92	+31	29.9	30.9	26.5	26.5	16.6	33.8	27.36	2.22	31.8	30.5	31.8	30.5	31.8	30.5	31.17	0.27	33.3	30.8	34.1	32.7	33.3	30.8	32.50	0.52	-	-
17-May-92	+32	29.2	34.5	31.6	25.4	18.8	32.4	28.65	2.14	29.8	30.6	29.8	30.6	29.8	30.6	30.24	0.16	31.1	30.9	33.0	37.0	31.1	30.9	32.34	0.90	-	-
24-May-92	+33	28.4	33.0	28.6	24.1	18.2	34.5	27.80	2.23	29.5	33.6	29.5	33.6	29.5	33.6	31.55	0.84	40.8	30.7	34.6	36.8	40.8	30.7	35.73	1.70	-	-
31-May-92	+34	30.7	**	29.6	28.5	19.0	36.2	28.80	2.49	33.9	34.9	33.9	34.9	33.9	34.9	34.36	0.20	36.6	31.6	35.5	37.3	36.6	31.6	34.87	0.97	-	-
07-Jun-92	+35	32.6	**	27.7	30.9	18.3	32.4	28.39	2.39	33.1	34.4	30.1	34.4	32.1	35.4	33.27	0.71	36.0	31.9	38.2	37.1	36.0	31.9	35.22	0.99	5.2	0.0479

Statistics = results of Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;
** = animal dead; the weeks when differences between the groups were significant are printed in bold faces.

Appendix Table 8.3.16: Serum Globulin Concentrations (g/l) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
Date	WPI	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	24.7	34.7	36.1	29.2	36.5	31.6	32.13	1.71	32.5	29.9	30.3	30.3	32.7	40.3	32.67	1.46	35.0	34.4	22.0	30.9	19.1	28.2	28.27	2.43	1.5	0.4699
08-Sep-91	-4	40.0	35.0	38.4	24.0	43.1	38.9	36.57	2.49	24.0	25.3	26.7	27.5	32.4	25.5	26.90	1.10	24.3	37.3	36.4	37.9	19.5	36.3	31.95	2.96	-	-
15-Sep-91	-3	28.4	28.4	25.8	28.3	34.6	28.0	28.92	1.10	29.9	28.5	23.1	30.2	33.3	29.5	29.08	1.25	19.8	28.1	34.7	36.3	33.4	26.5	29.80	2.32	-	-
22-Sep-91	-2	31.6	29.3	30.3	30.9	33.6	37.9	32.27	1.16	36.1	31.8	36.7	23.8	40.7	37.9	34.50	2.23	26.2	36.9	33.4	27.7	38.0	33.8	32.67	1.78	-	-
29-Sep-91	-1	40.9	27.4	32.2	25.9	31.4	36.0	32.30	2.06	20.6	27.5	33.3	30.6	37.4	29.5	29.82	2.11	35.6	29.9	29.6	30.5	30.9	26.3	30.47	1.12	-	-
06-Oct-91	0	30.6	29.1	41.8	33.9	11.7	40.7	31.30	4.06	41.8	49.8	32.1	39.0	27.2	35.8	37.62	2.93	29.1	29.2	32.7	34.6	34.9	20.0	30.08	2.07	2.4	0.3048
13-Oct-91	+1	33.2	39.7	43.7	28.1	33.4	45.5	37.27	2.53	38.5	28.5	38.3	29.7	44.3	33.8	35.52	2.23	23.3	37.9	34.4	32.0	33.1	33.7	32.40	1.82	-	-
20-Oct-91	+2	32.4	38.3	44.2	30.3	28.6	44.0	36.30	2.56	36.9	35.5	41.5	32.8	47.5	38.7	38.82	1.93	27.2	52.1	34.3	34.6	36.7	32.6	36.25	3.13	-	-
27-Oct-91	+3	27.3	28.4	29.3	25.3	27.7	34.6	28.77	1.18	28.7	27.9	28.9	23.7	35.7	32.3	29.53	1.52	21.4	42.0	28.1	35.9	34.0	26.2	31.27	2.77	-	-
03-Nov-91	+4	31.9	28.1	34.3	31.0	34.9	39.6	33.30	1.47	42.7	28.7	35.2	33.8	45.7	39.4	37.57	2.32	29.8	32.3	32.0	33.8	25.0	31.3	30.70	1.15	-	-
10-Nov-91	+5	21.5	24.1	25.0	23.0	26.7	32.0	25.38	1.38	26.4	24.2	29.3	27.3	28.3	36.5	28.67	1.57	23.0	39.5	36.1	38.3	26.1	32.3	32.55	2.51	4.4	0.1083
17-Nov-91	+6	40.0	34.1	38.3	33.5	35.4	34.0	35.88	0.99	42.6	38.5	32.8	37.0	33.8	35.8	36.75	1.32	31.3	41.3	36.1	34.4	32.2	36.2	35.25	1.33	-	-
24-Nov-91	+7	28.8	37.2	33.7	33.0	30.3	33.1	32.68	1.09	32.8	29.3	21.6	23.4	31.4	29.3	27.97	1.67	27.7	28.4	24.4	30.1	23.2	29.6	27.23	1.05	-	-
01-Dec-91	+8	26.4	27.7	38.8	31.9	29.3	31.9	31.00	1.64	31.7	33.0	31.3	32.2	39.2	41.8	34.87	1.67	28.4	38.4	35.7	30.8	26.5	34.6	32.40	1.71	-	-
08-Dec-91	+9	32.7	37.6	36.1	38.2	38.7	40.9	37.37	1.03	39.9	38.8	31.9	28.8	42.3	46.4	38.02	2.44	25.5	42.6	30.4	29.9	23.2	41.9	32.25	3.06	-	-
15-Dec-91	+10	38.1	45.5	39.4	40.8	43.6	44.0	41.90	1.08	43.8	34.7	37.9	32.1	48.3	38.5	39.22	2.22	36.3	48.5	42.0	34.6	29.7	42.1	38.87	2.48	1.5	0.4758
22-Dec-91	+11	23.6	35.5	37.1	41.7	39.5	40.0	36.23	2.45	33.8	25.5	32.9	25.1	37.9	37.5	32.12	2.10	26.1	35.9	27.0	30.5	15.5	36.4	28.57	2.88	-	-
29-Dec-91	+12	32.1	23.9	28.6	26.3	45.3	44.0	33.37	3.41	34.6	32.8	32.7	27.8	35.7	42.7	34.38	1.82	27.2	40.5	33.1	33.9	18.2	39.1	32.00	3.08	-	-
05-Jan-92	+13	37.7	37.3	46.0	43.3	60.6	50.2	45.85	3.26	39.7	39.6	32.8	38.0	40.3	44.9	39.22	1.46	26.5	39.8	32.7	33.5	15.8	37.3	30.93	3.24	-	-
12-Jan-92	+14	30.6	31.8	42.4	38.3	47.4	45.8	39.38	2.64	40.9	29.4	28.1	28.5	31.5	32.9	31.88	1.78	23.6	38.6	27.2	29.7	30.3	25.2	29.10	1.98	-	-
19-Jan-92	+15	25.9	26.4	37.7	32.0	48.3	46.4	36.12	3.62	38.0	27.9	30.5	24.7	30.5	32.2	30.63	1.66	25.5	34.6	22.2	29.0	29.6	28.4	28.22	1.55	2.5	0.2908
26-Jan-92	+16	27.6	31.1	47.3	48.8	54.8	44.7	42.38	3.98	38.7	36.6	31.0	34.4	34.5	34.2	34.90	0.96	27.1	40.5	31.1	31.6	21.0	31.2	30.42	2.38	-	-
02-Feb-92	+17	28.4	31.7	35.0	39.8	46.5	45.2	37.78	2.73	41.0	43.3	33.9	28.7	37.8	41.3	37.67	2.05	31.6	44.7	31.7	38.3	26.5	37.3	35.00	2.39	-	-
09-Feb-92	+18	36.8	31.4	36.9	35.5	46.3	40.7	37.93	1.89	40.2	35.7	37.5	28.9	46.0	33.5	36.99	2.18	29.3	46.4	29.8	38.5	24.7	36.9	34.26	2.93	-	-
16-Feb-92	+19	29.3	28.0	36.8	30.7	30.0	37.7	32.10	1.54	36.4	32.6	22.9	24.9	36.2	37.0	31.68	2.33	28.6	41.8	26.4	26.9	25.2	35.6	30.76	2.45	-	-
23-Feb-92	+20	28.3	32.8	36.6	30.3	38.1	45.7	35.29	2.34	40.5	39.3	33.5	32.0	37.0	37.1	36.57	1.22	34.0	49.2	30.1	32.5	27.1	34.6	34.58	2.86	1.5	0.4843
01-Mar-92	+21	24.0	25.5	25.4	29.6	32.6	43.0	30.02	2.65	28.5	35.4	24.1	25.2	33.2	33.5	29.98	1.76	30.2	46.7	23.5	32.3	22.9	35.3	31.81	3.27	-	-
08-Mar-92	+22	33.7	28.9	29.2	23.8	37.8	36.3	31.62	1.97	30.6	35.7	28.9	29.5	36.1	36.7	32.92	1.34	26.2	44.4	28.7	31.7	22.7	40.4	32.35	3.14	-	-
15-Mar-92	+23	32.0	34.8	39.0	30.8	32.6	41.7	35.15	1.60	40.9	32.9	36.4	27.5	40.1	33.5	35.22	1.87	32.9	45.4	33.2	34.9	26.4	37.7	35.10	2.35	-	-
22-Mar-92	+24	30.4	33.9	32.0	29.7	27.8	42.9	32.82	2.00	30.8	31.2	39.5	32.7	46.3	41.0	36.91	2.36	31.4	42.6	34.8	39.2	27.3	36.9	35.39	2.05	-	-
29-Mar-92	+25	28.9	25.4	27.7	31.3	35.9	40.2	31.56	2.06	31.8	31.9	28.9	26.3	37.5	38.8	32.51	1.81	31.0	41.0	33.2	29.2	28.6	32.7	32.61	1.67	0.5	0.7741
05-Apr-92	+26	26.5	25.7	28.6	27.8	30.4	32.9	28.65	0.99	29.9	32.4	29.2	33.6	32.1	32.5	31.62	0.63	26.1	35.6	26.1	25.7	28.9	32.5	29.15	1.52	-	-
12-Apr-92	+27	27.3	29.4	31.0	30.6	27.5	42.9	31.46	2.16	30.4	41.2	30.2	34.1	34.1	53.0	37.18	3.25	32.2	41.1	27.2	37.2	25.8	35.2	33.11	2.20	-	-
19-Apr-92	+28	26.5	31.9	30.6	27.8	27.7	39.2	30.61	1.74	36.0	33.7	36.2	33.7	35.5	38.1	35.54	0.63	30.3	41.4	29.0	35.1	28.2	37.5	33.58	1.98	-	-
26-Apr-92	+29	34.1	27.1	39.4	27.0	32.4	45.6	34.28	2.70	29.5	38.7	44.1	38.2	32.6	45.0	38.04	2.29	31.9	46.6	26.8	38.9	32.4	39.4	35.99	2.63	-	-
03-May-92	+30	25.6	26.5	29.8	27.5	29.9	35.4	29.10	1.31	28.4	29.6	32.3	33.0	30.8	34.4	31.42	0.83	30.6	39.3	30.3	33.2	32.9	35.5	33.62	1.25	5.4	0.0667
10-May-92	+31	28.0	25.2	27.9	27.6	23.2	32.1	27.32	1.12	25.4	31.2	32.6	34.5	30.8	43.2	32.95	2.19	31.2	40.1	27.5	36.0	33.6	30.0	33.06	1.68	-	-
17-May-92	+32	26.2	24.0	23.8	28.6	20.5	29.6	25.45	1.27	29.0	29.9	29.1	25.5	38.1	38.5	31.68	1.99	34.9	34.5	30.1	29.1	33.4	29.6	31.93	0.98	-	-
24-May-92	+33	22.7	21.7	19.2	25.4	26.5	29.1	24.10	1.34	24.4	23.9	29.9	27.8	32.9	28.2	27.87	1.26	24.3	38.3	32.3	25.3	20.3	34.0	29.08	2.55	-	-
31-May-92	+34	21.6	**	23.6	21.2	22.7	26.6	23.16	0.86	22.0	27.5	26.1	20.2	31.8	33.1	26.78	1.93	23.4	36.5	28.2	27.8	28.7	40.2	30.80	2.33	-	-
07-Jun-92	+35	23.5	**	28.2	23.5	25.4	28.9	25.91	1.02	26.6	23.4	30.7	20.7	30.8	26.4	26.43	1.49	25.9	29.0	29.3	26.0	30.3	33.6	29.02	1.08	2.7	0.2642

Statistics = results of Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;.
**** = animal dead; the weeks when differences between the groups were significant are printed in bold faces.**

Appendix Table 8.3.17: Serum Albumin to Serum Globulin Ratios of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
Date	WPI	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	1.4	0.9	0.9	1.0	1.0	1.0	1.04	0.07	0.9	1.1	1.1	1.1	0.9	0.8	0.99	0.05	0.8	1.1	1.7	1.1	1.5	1.3	1.25	0.12	3.1	0.2087
08-Sep-91	-4	0.7	0.9	0.8	1.4	0.7	0.9	0.91	0.10	1.4	1.4	1.2	1.3	1.0	1.4	1.27	0.05	1.4	0.9	0.9	0.8	1.7	0.9	1.10	0.14	-	-
15-Sep-91	-3	1.0	1.1	1.3	1.2	0.9	1.2	1.11	0.06	1.2	1.2	1.5	1.2	1.1	1.2	1.24	0.06	1.8	1.2	0.9	0.8	1.0	1.2	1.15	0.12	-	-
22-Sep-91	-2	0.9	1.1	1.1	1.1	0.9	0.9	1.01	0.04	0.9	1.1	0.9	1.4	0.8	0.9	1.02	0.08	1.3	0.8	1.0	1.1	0.9	0.9	1.00	0.06	-	-
29-Sep-91	-1	0.7	1.1	1.0	1.3	1.0	0.9	1.00	0.07	1.6	1.2	1.0	1.1	0.9	1.2	1.17	0.09	0.9	1.0	1.1	1.0	1.0	1.2	1.05	0.04	-	-
06-Oct-91	0	1.0	1.1	0.8	1.0	2.8	0.8	1.27	0.29	0.8	0.6	1.1	0.8	1.3	0.8	0.89	0.09	1.1	1.3	1.0	1.0	1.0	1.9	1.21	0.14	2.9	0.2291
13-Oct-91	+1	1.0	0.8	0.8	1.3	1.0	0.7	0.93	0.07	0.9	1.2	0.9	1.2	0.8	1.1	1.02	0.07	1.5	0.9	1.0	1.1	1.0	1.0	1.10	0.08	-	-
20-Oct-91	+2	1.0	0.8	0.7	1.2	1.2	0.8	0.97	0.08	0.9	0.9	0.8	1.0	0.7	0.8	0.87	0.03	1.3	0.7	1.0	1.1	1.0	1.1	1.03	0.08	-	-
27-Oct-91	+3	1.2	1.1	1.2	1.3	1.2	1.0	1.17	0.04	1.2	1.2	1.2	1.4	1.0	1.0	1.19	0.06	1.6	0.7	1.3	1.0	1.0	1.2	1.14	0.11	-	-
03-Nov-91	+4	0.9	1.5	1.2	1.0	0.9	0.7	1.03	0.10	0.6	1.1	0.9	1.0	0.8	0.8	0.89	0.06	1.0	1.1	1.2	1.0	1.1	1.2	1.10	0.03	-	-
10-Nov-91	+5	1.4	1.4	1.4	1.6	1.4	1.1	1.40	0.06	1.4	1.4	1.3	1.2	1.3	0.9	1.24	0.07	1.5	0.8	0.9	0.8	1.2	1.1	1.05	0.11	5.1	0.0770
17-Nov-91	+6	0.8	0.9	0.9	1.0	1.0	1.0	0.91	0.03	0.8	0.8	1.0	0.9	1.0	0.9	0.92	0.04	0.9	0.8	1.0	1.0	1.0	0.8	0.93	0.04	-	-
24-Nov-91	+7	1.1	0.9	1.0	0.9	1.1	1.1	1.03	0.04	1.0	1.1	1.8	1.5	1.2	1.2	1.30	0.10	1.1	1.1	1.4	1.1	1.3	1.0	1.16	0.06	-	-
01-Dec-91	+8	1.3	1.2	0.8	1.0	1.2	1.2	1.10	0.07	1.1	1.0	1.1	1.0	0.9	0.9	0.99	0.04	1.1	0.8	0.9	1.0	1.2	1.0	1.01	0.05	-	-
08-Dec-91	+9	1.0	0.9	0.9	0.8	0.9	0.9	0.90	0.03	0.8	0.9	1.1	1.2	0.9	0.7	0.94	0.06	1.5	0.7	1.2	1.2	1.6	0.7	1.16	0.14	-	-
15-Dec-91	+10	0.8	0.8	0.9	0.9	0.8	0.8	0.83	0.02	0.8	1.1	0.9	1.2	0.7	1.0	0.92	0.07	1.0	0.7	0.8	1.0	1.2	0.8	0.92	0.06	1.3	0.5239
22-Dec-91	+11	1.5	0.9	0.9	0.7	0.9	0.8	0.96	0.11	1.0	1.4	1.0	1.4	0.9	0.9	1.11	0.09	1.3	0.9	1.2	1.3	2.2	0.9	1.30	0.17	-	-
29-Dec-91	+12	1.1	1.7	1.3	1.5	0.7	0.9	1.18	0.13	1.1	1.1	1.2	1.3	1.1	0.8	1.09	0.06	1.4	0.8	1.1	1.0	2.1	0.9	1.22	0.18	-	-
05-Jan-92	+13	0.8	1.0	0.8	0.8	0.4	0.7	0.76	0.07	0.9	0.8	1.1	0.9	0.9	0.7	0.91	0.05	1.5	0.9	1.1	1.1	2.5	0.9	1.31	0.23	-	-
12-Jan-92	+14	1.1	1.0	0.7	0.8	0.6	0.8	0.83	0.07	0.9	1.2	1.3	1.2	1.1	1.0	1.11	0.05	1.5	0.9	1.4	1.2	1.2	1.4	1.28	0.08	-	-
19-Jan-92	+15	1.2	1.3	0.9	1.0	0.5	2.1	1.18	0.20	0.9	1.3	1.2	1.5	1.2	1.1	1.19	0.06	1.5	1.1	1.7	1.3	0.8	1.3	1.26	0.11	0.9	0.6476
26-Jan-92	+16	1.3	1.1	0.7	0.6	0.4	0.8	0.82	0.13	0.9	1.0	1.2	1.1	1.1	1.1	1.07	0.03	1.4	0.9	1.2	1.2	1.8	1.1	1.27	0.11	-	-
02-Feb-92	+17	1.2	1.1	1.0	0.8	0.5	0.8	0.88	0.09	0.9	0.8	1.1	1.3	1.0	0.9	1.00	0.06	1.2	0.8	1.3	1.0	1.4	1.0	1.12	0.08	-	-
09-Feb-92	+18	0.9	1.0	0.9	0.9	0.4	0.8	0.80	0.08	0.8	1.0	0.8	1.2	0.7	1.1	0.92	0.08	1.2	0.7	1.4	0.8	1.4	0.9	1.06	0.12	-	-
16-Feb-92	+19	1.2	1.3	0.9	1.2	0.9	1.0	1.07	0.06	1.1	1.1	1.7	1.5	1.1	1.0	1.24	0.10	1.3	0.9	1.5	1.5	1.5	1.0	1.29	0.10	-	-
23-Feb-92	+20	1.2	1.0	0.9	1.0	0.5	0.7	0.88	0.10	0.9	0.9	1.0	1.1	0.9	0.9	0.96	0.03	1.1	0.7	1.2	1.1	1.4	1.0	1.08	0.09	3.1	0.2096
01-Mar-92	+21	1.5	1.4	1.3	1.1	0.7	0.8	1.13	0.12	1.3	1.0	1.6	1.3	1.1	1.0	1.22	0.09	1.3	0.7	1.6	1.2	1.8	1.0	1.26	0.15	-	-
08-Mar-92	+22	0.9	1.1	1.0	1.4	0.5	1.0	0.98	0.11	1.2	0.9	1.2	1.1	1.0	0.9	1.07	0.05	1.4	0.7	1.3	1.2	1.7	0.8	1.20	0.14	-	-
15-Mar-92	+23	1.0	0.9	0.6	1.0	0.6	0.8	0.80	0.06	0.8	1.2	0.9	1.4	0.8	1.1	1.01	0.09	1.0	0.7	1.1	1.1	1.3	0.9	1.02	0.07	-	-
22-Mar-92	+24	1.1	0.9	0.8	1.0	0.6	0.8	0.86	0.05	1.1	1.1	0.8	1.0	0.7	0.8	0.91	0.06	1.2	0.8	1.1	0.9	1.4	0.9	1.05	0.08	-	-
29-Mar-92	+25	1.2	1.4	1.1	1.0	0.5	0.9	0.99	0.11	1.1	1.1	1.2	1.3	0.9	0.9	1.08	0.06	1.1	0.9	1.1	1.3	1.2	1.1	1.11	0.05	0.3	0.8427
05-Apr-92	+26	1.3	1.3	1.3	1.5	0.8	1.3	1.24	0.09	1.3	1.3	1.3	1.3	1.0	1.1	1.22	0.04	1.3	1.3	1.3	1.4	1.5	1.3	1.35	0.03	-	-
12-Apr-92	+27	1.1	1.1	0.9	0.9	0.6	0.8	0.90	0.07	1.1	0.7	1.1	0.9	1.0	0.6	0.89	0.08	1.1	0.8	1.2	0.9	1.4	0.9	1.04	0.08	-	-
19-Apr-92	+28	1.3	1.0	0.9	1.0	0.7	0.9	0.97	0.08	0.9	1.0	0.9	1.0	0.9	0.9	0.93	0.02	1.1	0.8	1.2	1.0	1.4	0.9	1.07	0.09	-	-
26-Apr-92	+29	0.9	1.1	0.8	1.0	0.5	0.7	0.84	0.08	1.1	0.8	0.8	0.8	1.0	0.7	0.86	0.07	1.1	0.7	1.3	0.9	1.0	1.2	1.03	0.08	-	-
03-May-92	+30	1.2	1.1	0.9	0.9	0.9	1.1	1.02	0.05	1.2	1.2	0.9	0.8	1.1	0.9	1.03	0.06	1.0	0.8	1.1	1.1	1.3	0.9	1.05	0.06	0.0	0.9953
10-May-92	+31	1.1	1.2	1.0	1.0	0.7	1.1	1.00	0.06	1.3	1.0	1.0	0.9	1.0	0.7	0.97	0.07	1.1	0.8	1.2	0.9	1.0	1.0	1.00	0.06	-	-
17-May-92	+32	1.1	1.4	1.3	0.9	0.9	1.1	1.13	0.08	1.0	1.0	1.0	1.2	0.8	0.8	0.98	0.06	0.9	0.9	1.1	1.3	0.9	1.0	1.02	0.06	-	-
24-May-92	+33	1.3	1.5	1.5	1.0	1.1	1.2	1.25	0.08	1.2	1.4	1.0	1.2	0.9	1.2	1.15	0.07	1.7	0.8	1.1	1.5	2.0	0.9	1.32	0.18	-	-
31-May-92	+34	1.4	**	1.3	1.3	0.8	1.4	1.24	0.09	1.5	1.3	1.3	1.7	1.1	1.1	1.33	0.10	1.6	0.9	1.3	1.3	1.3	0.8	1.18	0.11	-	-
07-Jun-92	+35	1.4	**	1.0	1.3	0.7	1.1	1.11	0.11	1.2	1.5	1.0	1.7	1.0	1.3	1.29	0.10	1.4	1.1	1.3	1.4	1.2	1.0	1.23	0.07	0.9	0.6262

Statistics = results of Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;.
**** = animal dead; the weeks when differences between the groups were significant are printed in bold faces.**

Appendix Table 8.3.18: Serum Glutamate Dehydrogenase (U/l) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
Date	WPI	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	16	16	19	11	18	17	16.3	1.1	15	23	15	13	20	19	17.4	1.5	11	14	14	21	18	18	15.9	1.4	0.5	0.7656
08-Sep-91	-4	15	16	18	20	16	17	17.2	0.7	19	16	16	19	17	18	17.5	0.5	13	18	14	17	14	18	15.7	1.0	-	-
15-Sep-91	-3	19	21	15	22	15	13	17.4	1.4	20	21	16	19	11	16	17.2	1.4	17	11	11	14	16	15	14.0	1.0	-	-
22-Sep-91	-2	24	15	16	18	15	17	17.5	1.2	16	16	19	11	18	17	16.3	1.1	15	16	18	20	16	17	17.2	0.7	-	-
29-Sep-91	-1	11	14	24	21	18	18	17.5	1.7	17	29	15	16	19	13	18.1	2.2	14	16	15	15	17	13	15.1	0.5	-	-
06-Oct-91	0	15	23	15	13	20	19	17.4	1.5	16	16	16	17	16	17	16.1	0.3	12	14	10	19	14	16	14.2	1.2	3.4	0.1808
13-Oct-91	+1	16	19	15	26	27	21	20.7	1.8	12	27	27	14	15	19	19.1	2.5	17	17	15	18	12	14	15.6	0.9	-	-
20-Oct-91	+2	11	20	18	18	16	14	16.2	1.2	14	16	16	15	23	18	16.9	1.2	12	18	15	14	14	14	14.5	0.8	-	-
27-Oct-91	+3	13	19	18	23	14	15	16.9	1.5	13	16	18	23	18	16	17.3	1.2	19	16	16	19	17	18	17.5	0.5	0.4	0.8189
03-Nov-91	+4	29	31	27	48	79	32	41.0	7.5	51	49	22	24	18	18	30.4	5.8	12	14	10	15	16	14	13.5	0.8	12.1	0.0023
10-Nov-91	+5	183	91	119	37	173	91	115.6	20.6	119	165	83	29	29	100	87.4	19.7	15	13	11	14	17	16	14.2	0.8	11.9	0.0026
17-Nov-91	+6	73	45	84	89	52	47	65.0	7.3	20	38	34	39	21	68	36.7	6.5	16	22	14	8	26	15	16.6	2.3	-	-
24-Nov-91	+7	37	35	47	26	33	35	35.5	2.5	27	25	34	38	37	40	33.6	2.2	14	14	17	12	15	13	14.2	0.7	-	-
01-Dec-91	+8	32	45	42	35	44	30	38.1	2.4	37	27	27	26	25	21	27.3	2.0	19	17	18	16	18	19	18.1	0.4	-	-
08-Dec-91	+9	32	55	35	23	23	33	33.2	4.4	33	33	31	27	12	31	27.9	2.9	13	13	21	17	17	12	15.5	1.3	-	-
15-Dec-91	+10	73	49	192	37	91	146	97.9	22.3	12	100	137	155	73	28	84.4	21.4	13	18	14	17	14	18	15.7	1.0	8.3	0.0160
22-Dec-91	+11	106	18	228	46	246	265	151.6	40.4	91	164	46	84	59	18	77.1	18.7	16	18	14	17	12	16	15.7	0.9	-	-
29-Dec-91	+12	89	73	146	18	155	109	98.3	18.8	119	274	77	82	88	29	111.4	31.5	17	12	12	17	14	16	14.7	0.8	-	-
05-Jan-92	+13	27	151	66	37	93	100	78.9	17.0	82	107	59	96	79	64	81.0	6.8	14	16	16	11	9	16	13.8	1.1	-	-
12-Jan-92	+14	46	173	29	155	64	128	99.1	22.7	38	46	19	137	110	119	77.8	18.5	9	16	10	15	14	19	13.7	1.3	-	-
19-Jan-92	+15	37	64	46	100	137	100	80.7	14.3	119	57	100	98	79	37	81.8	11.4	12	14	9	16	18	16	14.2	1.2	11.4	0.0033
26-Jan-92	+16	27	67	73	57	87	68	63.2	7.5	77	97	88	59	46	67	72.4	7.0	14	13	14	19	9	17	14.3	1.3	-	-
02-Feb-92	+17	83	183	128	64	86	37	96.6	19.2	29	49	65	37	58	101	56.4	9.5	12	19	17	19	15	17	16.6	1.1	-	-
09-Feb-92	+18	18	82	128	119	46	91	80.6	15.7	49	64	70	19	19	91	52.1	10.8	9	22	27	16	16	18	18.0	2.3	-	-
16-Feb-92	+19	37	82	46	18	265	18	77.6	35.3	73	29	39	27	55	110	55.6	11.8	17	18	16	18	16	13	16.6	0.7	-	-
23-Feb-92	+20	68	46	64	91	37	91	66.0	8.4	37	77	29	37	55	77	51.7	7.9	12	15	17	11	20	9	14.0	1.5	12.0	0.0025
01-Mar-92	+21	51	39	151	37	35	58	61.9	16.7	21	32	64	27	46	40	38.2	5.7	15	17	13	15	17	7	13.9	1.5	-	-
08-Mar-92	+22	46	27	38	46	29	19	34.2	4.0	19	38	16	18	75	37	33.8	8.3	12	11	14	17	18	15	14.5	1.1	-	-
15-Mar-92	+23	76	47	29	73	73	27	54.3	8.4	32	27	66	35	38	31	38.2	5.2	14	12	12	14	17	18	14.4	0.9	-	-
22-Mar-92	+24	55	64	26	20	64	18	41.2	8.2	55	37	25	31	16	29	32.1	4.9	14	17	9	18	16	9	13.8	1.5	-	-
29-Mar-92	+25	73	119	73	100	18	64	74.5	12.8	29	82	55	91	20	35	52.0	10.9	12	17	9	9	16	17	13.3	1.4	11.8	0.0027
05-Apr-92	+26	37	37	27	73	40	43	42.7	5.9	51	46	22	29	31	23	33.6	4.5	15	18	16	18	17	12	16.0	0.9	-	-
12-Apr-92	+27	82	22	27	64	64	29	48.1	9.3	40	37	37	37	22	11	30.4	4.3	22	27	13	19	16	19	19.3	1.9	-	-
19-Apr-92	+28	47	26	13	20	29	24	26.5	4.4	23	26	15	22	22	16	20.5	1.6	20	18	19	12	18	15	17.0	1.1	-	-
26-Apr-92	+29	27	46	26	27	27	19	28.7	3.3	17	26	27	19	35	15	23.0	2.8	18	14	17	16	15	16	16.0	0.6	-	-
03-May-92	+30	37	37	20	46	37	18	32.5	4.1	23	18	14	18	16	18	18.1	1.1	16	16	16	16	22	13	16.3	1.1	9.2	0.0101
10-May-92	+31	31	47	22	11	16	20	24.6	4.8	16	19	18	18	17	24	18.6	1.0	14	16	19	13	18	20	16.4	1.1	1.9	0.3823
17-May-92	+32	13	13	14	12	42	20	18.8	4.4	11	14	18	21	14	11	14.7	1.5	19	14	13	17	15	15	15.3	0.9	0.1	0.9693
24-May-92	+33	18	24	15	14	26	14	18.4	1.9	17	13	14	29	11	15	16.5	2.4	12	24	17	18	17	24	18.7	1.7	-	-
31-May-92	+34	18	**	16	16	18	18	17.5	0.4	9	16	27	14	11	16	15.6	2.4	13	24	14	22	20	11	17.2	2.0	-	-
07-Jun-92	+35	23	**	18	11	19	22	18.6	1.9	15	16	11	19	16	17	15.8	1.0	14	11	24	24	7	13	15.3	2.6	0.2	0.5371

Statistics = results of Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;
**** = animal dead; the weeks when differences between the groups were significant are printed in bold faces.**

Appendix Table 8.3.19: Serum Gamma Glutamyl Transpeptidase (U/l) of goats
Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)								Infected goats (Group B)								Uninfected goats (Group C)								Statistics	
Date	WPI	31	32	33	34	35	36	Mean	SE	41	42	43	44	45	46	Mean	SE	51	52	53	54	55	56	Mean	SE	KW	P
01-Sep-91	-5	21	22	34	27	14	27	24.1	2.5	16	23	25	22	26	27	23.2	1.4	24	24	22	24	27	28	24.8	0.8	-	-
08-Sep-91	-4	30	31	32	34	31	33	32.0	0.6	23	35	33	35	33	35	32.2	1.7	34	32	33	30	34	32	32.5	0.5	-	-
15-Sep-91	-3	23	32	26	27	34	22	27.3	1.7	22	24	27	27	35	32	27.6	1.7	27	25	31	33	33	25	29.1	1.4	-	-
22-Sep-91	-2	33	27	32	25	26	28	28.5	1.1	26	23	35	25	28	28	27.4	1.5	28	25	26	34	24	24	26.6	1.4	-	-
29-Sep-91	-1	26	23	35	22	26	27	26.5	1.6	31	22	34	25	34	27	28.8	1.8	31	27	24	30	28	35	29.2	1.4	-	-
06-Oct-91	0	23	29	27	28	27	25	26.4	0.8	22	29	30	29	31	29	28.1	1.2	30	29	27	28	30	27	28.7	0.5	-	-
13-Oct-91	+1	29	26	31	32	31	25	28.8	1.0	28	26	32	26	34	31	29.5	1.1	32	31	35	31	26	32	31.0	1.1	-	-
20-Oct-91	+2	26	25	34	34	34	27	29.9	1.5	25	24	35	31	29	27	28.4	1.5	32	34	28	35	27	25	30.1	1.5	-	-
27-Oct-91	+3	26	24	36	34	32	26	29.8	1.9	25	31	31	32	35	28	30.2	1.3	32	28	31	28	29	31	29.7	0.6	-	-
03-Nov-91	+4	29	27	31	32	32	24	29.3	1.2	26	35	23	30	29	34	29.6	1.7	27	29	31	35	35	31	31.2	1.2	-	-
10-Nov-91	+5	30	29	30	35	36	28	31.3	1.3	28	27	27	37	30	34	30.5	1.6	30	28	34	31	23	23	28.0	1.6	-	-
17-Nov-91	+6	35	27	27	31	31	31	30.0	1.1	28	31	25	32	29	36	30.3	1.4	26	33	24	32	33	33	30.4	1.5	-	-
24-Nov-91	+7	34	29	29	25	23	30	28.4	1.4	28	35	27	29	31	38	31.2	1.7	27	35	33	26	26	35	30.2	1.6	-	-
01-Dec-91	+8	27	24	25	30	31	26	27.2	1.0	26	32	28	27	28	31	28.6	0.9	34	34	22	34	27	28	29.8	1.9	-	-
08-Dec-91	+9	30	25	31	36	27	27	29.6	1.4	23	31	27	26	31	38	29.4	2.0	35	34	32	27	24	22	28.9	2.0	-	-
15-Dec-91	+10	28	24	35	30	38	20	29.1	2.5	23	27	22	29	24	36	26.8	1.9	33	35	27	32	23	20	28.4	2.3	0.5	0.7801
22-Dec-91	+11	39	29	57	81	78	29	52.1	8.7	23	52	29	35	23	52	35.7	5.0	31	26	33	27	23	35	29.2	1.6	4.2	0.1222
29-Dec-91	+12	58	41	116	110	203	353	146.7	43.2	52	29	32	29	29	52	37.2	4.3	35	31	27	33	23	29	29.6	1.6	10.8	0.0046
05-Jan-92	+13	57	197	261	110	203	191	169.8	27.2	92	40	35	46	46	81	56.8	8.8	34	26	30	33	29	29	30.2	1.0	-	-
12-Jan-92	+14	64	93	69	93	64	243	104.2	25.9	75	88	99	98	66	35	77.0	9.1	35	29	29	35	35	23	31.0	1.8	-	-
19-Jan-92	+15	52	69	110	81	64	122	83.0	10.2	58	46	46	98	59	49	59.4	7.3	30	35	33	32	23	23	29.4	1.9	13.1	0.0014
26-Jan-92	+16	35	35	64	75	52	93	58.9	8.6	52	46	29	139	35	42	57.1	15.3	35	29	32	35	29	27	31.1	1.2	-	-
02-Feb-92	+17	41	41	69	75	98	64	64.6	8.2	41	52	41	75	86	46	56.8	7.2	25	27	33	30	32	26	28.9	1.2	-	-
09-Feb-92	+18	41	53	75	64	52	52	56.1	4.4	58	69	46	64	81	64	63.7	4.3	28	28	26	31	27	28	28.2	0.6	-	-
16-Feb-92	+19	39	55	64	81	86	62	64.5	6.5	54	91	32	91	51	59	62.9	8.8	22	21	33	29	27	29	26.9	1.7	-	-
23-Feb-92	+20	39	65	29	52	116	46	57.8	11.5	35	52	46	110	46	75	60.8	10.3	35	31	29	27	31	33	30.9	1.0	8.6	0.0133
01-Mar-92	+21	81	69	35	41	89	41	59.1	8.7	69	81	41	87	52	46	62.7	7.1	29	23	24	30	27	33	27.7	1.4	-	-
08-Mar-92	+22	58	162	29	17	64	55	64.2	19.1	81	52	64	98	35	29	59.8	10.0	23	26	32	32	24	32	28.1	1.6	-	-
15-Mar-92	+23	64	69	145	64	116	62	86.5	13.1	49	87	69	81	41	32	59.7	8.4	26	26	35	30	33	29	29.8	1.3	-	-
22-Mar-92	+24	41	93	110	76	68	53	73.5	9.5	43	39	53	58	46	37	46.0	3.0	31	25	27	32	25	26	27.5	1.2	-	-
29-Mar-92	+25	41	156	46	87	58	110	83.0	16.6	47	41	41	29	46	66	44.9	4.5	32	32	27	35	23	27	29.4	1.6	11.3	0.0035
05-Apr-92	+26	93	179	64	87	46	29	83.0	19.8	35	35	39	43	56	47	42.5	3.0	32	32	36	33	35	27	32.4	1.1	-	-
12-Apr-92	+27	98	116	46	75	41	41	69.5	12.1	55	53	56	46	55	54	53.1	1.4	26	27	26	33	28	35	29.1	1.5	-	-
19-Apr-92	+28	52	122	61	101	59	81	79.3	10.2	46	46	46	40	72	23	45.6	6.0	24	34	26	31	26	35	29.2	1.7	-	-
26-Apr-92	+29	42	41	52	127	91	122	79.0	14.8	52	46	35	47	87	29	49.4	7.6	29	33	31	35	31	24	30.3	1.4	9.9	0.0071
03-May-92	+30	81	23	56	46	69	145	70.1	15.5	17	26	52	47	98	64	50.8	10.8	29	28	34	27	27	26	28.6	1.0	3.7	0.1563
10-May-92	+31	61	58	46	58	17	151	65.1	16.7	58	29	64	52	52	17	45.4	6.7	30	27	27	28	27	35	29.0	1.1	-	-
17-May-92	+32	49	41	35	94	110	75	67.1	11.4	58	41	81	37	29	17	43.9	8.4	27	25	25	29	27	23	26.1	0.8	-	-
24-May-92	+33	69	41	29	75	41	57	52.0	6.8	46	26	35	35	81	87	51.6	9.7	32	27	28	27	32	23	28.1	1.3	-	-
31-May-92	+34	37	**	64	41	52	35	46.8	5.7	46	35	81	37	23	23	41.0	8.0	32	35	23	30	32	30	30.1	1.5	-	-
07-Jun-92	+35	47	**	56	75	52	42	54.3	5.0	52	23	58	58	58	22	45.2	6.6	26	29	27	29	28	26	27.6	0.5	5.0	0.0818

Statistics = results of Kruskal-Wallis one-way analysis of variance; KW = Kruskal-Wallis statistic; P = significance (probability); - = analysis not done;
**** = animal dead; the weeks when differences between the groups were significant are printed in bold faces.**

Appendix Table 8.3.20: Results of agar gel precipitation tests on the sera of goats Experiment 3: Experimental infection of Nepalese hill goats with *Fasciola gigantica*

		Infected goats (Group A)						Infected goats (Group B)						Uninfected goats (Group C)					
Date	WPI	31	32	33	34	35	36	41	42	43	44	45	46	51	52	53	54	55	56
01-Sep-91	-5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
08-Sep-91	-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15-Sep-91	-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22-Sep-91	-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29-Sep-91	-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
06-Oct-91	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13-Oct-91	+1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-Oct-91	+2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27-Oct-91	+3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
03-Nov-91	+4	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
10-Nov-91	+5	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
17-Nov-91	+6	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
24-Nov-91	+7	-	+	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-
01-Dec-91	+8	-	+	+	+	+	-	-	+	+	+	-	+	-	-	-	-	-	-
08-Dec-91	+9	-	+	+	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-
15-Dec-91	+10	+	+	+	+	+	+	-	+	+	-	-	+	-	-	-	-	-	-
22-Dec-91	+11	-	+	+	+	+	-	-	+	-	-	+	+	-	-	-	-	-	-
29-Dec-91	+12	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
05-Jan-92	+13	+	+	+	+	+	+	-	-	+	-	+	+	-	-	-	-	-	-
12-Jan-92	+14	-	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
19-Jan-92	+15	-	+	+	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-
26-Jan-92	+16	-	+	+	+	+	-	-	+	+	-	+	+	-	-	-	-	-	-
02-Feb-92	+17	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-
09-Feb-92	+18	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
16-Feb-92	+19	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
23-Feb-92	+20	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
01-Mar-92	+21	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
08-Mar-92	+22	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
15-Mar-92	+23	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
22-Mar-92	+24	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
29-Mar-92	+25	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
05-Apr-92	+26	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
12-Apr-92	+27	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
19-Apr-92	+28	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
26-Apr-92	+29	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
03-May-92	+30	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
10-May-92	+31	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
17-May-92	+32	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
24-May-92	+33	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
31-May-92	+34	-	**	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
07-Jun-92	+35	-	**	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = positive for precipitin lines; - = negative for precipitin lines; ** = goat dead.

Appendix Table 8.4.1
Liveweights (kg) of the individual animals
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	t	P
03-Jan-91	-6	87	96	72	108	96	87	109	116	96.4	4.8	93	86	63	116	98	84	80	132	94.0	7.1	-	-
09-Jan-91	-5	84	92	72	108	93	88	112	116	95.6	5.0	102	86	61	112	96	83	80	133	94.1	7.3	-	-
16-Jan-91	-4	87	92	75	109	93	90	108	116	96.3	4.5	101	90	64	116	97	94	80	128	96.3	6.6	-	-
23-Jan-91	-3	91	96	79	115	99	90	112	117	99.9	4.5	106	92	65	121	101	90	85	137	99.6	7.4	-	-
30-Jan-91	-2	92	98	79	117	100	91	112	123	101.5	4.9	108	95	67	121	104	91	85	136	100.9	7.1	-	-
06-Feb-91	-1	93	99	81	116	100	93	112	125	102.4	4.7	108	97	68	123	106	92	88	132	101.8	6.7	-	-
13-Feb-91	0	97	104	82	120	105	97	117	129	106.4	5.0	113	97	70	128	110	93	93	137	105.1	7.1	0.15	0.8831
20-Feb-91	+1	96	100	81	117	100	97	116	124	103.9	4.7	112	97	68	127	107	93	92	137	104.1	7.2	-	-
27-Feb-91	+2	95	97	80	114	99	94	113	122	101.8	4.5	109	97	67	117	106	92	88	136	101.5	6.8	-	-
06-Mar-91	+3	90	89	77	109	106	90	109	119	98.6	4.7	109	87	66	122	102	87	95	131	99.9	6.9	-	-
13-Mar-91	+4	98	102	85	119	102	96	117	127	105.8	4.6	117	98	71	127	112	95	95	137	106.5	6.9	0.08	0.9339
20-Mar-91	+5	97	98	85	117	107	95	113	131	105.4	4.8	116	98	68	129	104	94	94	145	106.0	7.9	-	-
27-Mar-91	+6	97	100	85	121	100	96	115	126	105.0	4.7	117	100	70	133	107	95	93	137	106.5	7.3	-	-
03-Apr-91	+7	100	99	86	122	102	94	116	135	106.8	5.4	118	99	73	135	113	96	97	137	108.5	7.1	-	-
10-Apr-91	+8	97	97	85	122	97	90	112	133	104.1	5.5	116	105	69	135	109	93	94	138	107.4	7.5	0.35	0.7280
17-Apr-91	+9	100	98	88	120	100	93	111	134	105.5	5.0	119	107	71	138	111	96	98	141	110.1	7.6	-	-
24-Apr-91	+10	103	98	86	122	100	89	118	135	106.4	5.6	120	108	72	138	111	98	98	142	110.9	7.5	-	-
01-May-91	+11	98	93	81	116	98	86	111	136	102.4	5.9	118	105	73	133	108	98	100	142	109.6	7.1	-	-
08-May-91	+12	95	90	80	114	100	86	109	126	100.0	5.1	120	112	74	135	108	96	100	144	111.1	7.4	1.24	0.2371
15-May-91	+13	96	89	78	115	100	86	108	125	99.6	5.2	121	112	74	140	112	99	102	146	113.3	7.6	-	-
22-May-91	+14	92	**	78	109	97	86	100	120	97.4	4.9	120	111	74	134	109	98	100	144	110.9	7.0	-	-
29-May-91	+15	92	**	74	110	95	**	100	122	98.8	6.1	123	115	76	140	112	100	105	148	114.9	7.5	-	-
05-Jun-91	+16	95	**	75	109	89	**	98	121	97.8	5.9	124	111	79	140	112	100	107	141	114.3	6.8	1.76	0.1046
12-Jun-91	+17	94	**	73	107	84	**	94	123	95.8	6.5	127	111	79	144	114	100	109	145	116.1	7.3	-	-
19-Jun-91	+18	92	**	**	107	**	**	94	118	102.8	5.3	128	112	80	144	113	102	110	149	117.3	7.5	-	-
26-Jun-91	+19	93	**	**	110	**	**	96	119	104.5	5.3	133	116	83	148	115	102	115	152	120.5	7.6	-	-
03-Jul-91	+20	95	**	**	109	**	**	96	120	105.0	5.1	135	122	83	149	116	106	119	155	123.1	7.7	1.55	0.1520
10-Jul-91	+21	89	**	**	100	**	**	90	120	99.8	6.2	130	118	82	143	114	106	118	150	120.1	7.1	-	-
17-Jul-91	+22	95	**	**	113	**	**	91	124	105.8	6.7	140	115	84	153	119	110	120	160	125.1	8.2	-	-
24-Jul-91	+23	97	**	**	104	**	**	92	128	105.3	6.9	140	123	87	160	116	108	127	162	127.9	8.5	-	-
31-Jul-91	+24	97	**	**	115	**	**	89	130	107.8	8.0	143	124	88	160	118	110	126	162	128.9	8.3	1.60	0.1402
07-Aug-91	+25	91	**	**	100	**	**	90	116	99.3	5.2	141	118	85	162	111	110	128	160	126.9	8.8	-	-
14-Aug-91	+26	88	**	**	100	**	**	89	118	98.8	6.0	151	116	90	164	110	113	130	161	129.4	8.9	-	-
21-Aug-91	+27	**	**	**	105	**	**	**	129	117.0	8.5	152	118	91	168	113	112	132	169	131.9	9.4	-	-
28-Aug-91	+28	**	**	**	108	**	**	**	126	117.0	6.4	156	120	92	174	115	115	136	173	135.1	9.9	0.87	0.4109

Statistics = results of unpaired t test; t = t value; P = two-tailed P (probability) value; - = analysis not done; ** = animals dead.

Appendix Table 8.4.2
Regression statistics of the liveweight of individual animals
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

Group	Buffalo No	n	Line of best fit (Y=a+bX)	r2	P	Liveweight gain (kg/week)
	81	27	Y = 97.88-0.20X	0.198	0.020	-0.196
	83	14	Y = 101.69-0.66X	0.345	0.027	-0.664
	85	18	Y = 85.22-0.44X	0.267	0.028	-0.439
A	87	29	Y = 121.04-0.59X	0.540	<0.001	-0.589
	89	18	Y = 106.33-0.78X	0.560	<0.001	-0.777
	92	15	Y = 98.24-0.82X	0.729	<0.001	-0.822
	94	27	Y = 121.20-1.24X	0.872	<0.001	-1.239
	96	29	Y = 128.70-0.22X	0.106	0.085	-0.221
	Mean	22	Y = 107.54-0.62X	0.413	<0.001	-0.618
	82	27	Y = 105.85+1.33X	0.889	<0.001	+1.328
	84	14	Y = 90.96+1.42X	0.733	<0.001	+1.416
	86	18	Y = 65.95+0.63X	0.790	<0.001	+0.625
B	88	29	Y = 118.37+1.58X	0.887	<0.001	+1.580
	90	18	Y = 106.07+0.34X	0.310	0.016	+0.337
	91	15	Y = 90.70+0.52X	0.578	0.001	+0.521
	93	27	Y = 84.14+1.54X	0.914	<0.001	+1.537
	95	29	Y = 129.73+1.19X	0.846	<0.001	+1.186
	Mean	22	Y = 98.97+1.07X	0.728	<0.001	+1.066

Appendix Table 8.4.3
Comparisions of the slopes (b) of regression lines of liveweights against WPI
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

Comparision	d.f.	Slopes (b)	F value	t value	Probability (P)
Buffalo 81 vs 82	1	-0.20 vs +1.33	155.88	12.485	< 0.001
Buffalo 83 vs 84	1	-0.66 vs +1.42	33.12	5.755	< 0.001
Buffalo 85 vs 86	1	-0.44 vs +0.63	28.68	5.355	< 0.001
Buffalo 87 vs 88	1	-0.59 vs +1.58	208.35	14.434	< 0.001
Buffalo 89 vs 90	1	-0.78 vs +0.34	27.29	5.224	< 0.001
Buffalo 92 vs 91	1	-0.82 vs +0.52	52.09	7.217	< 0.001
Buffalo 94 vs 93	1	-1.24 vs +1.54	432.80	20.804	< 0.001
Buffalo 96 vs 95	1	-0.22 vs +1.19	79.96	8.942	< 0.001
Group A vs B	14	-0.62 vs +1.07	62.89	7.930	< 0.001

Appendix Table 8.4.4
Faecal Egg Counts (EPG) of Buffalo calves
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
08-Jan-91	-5	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
16-Jan-91	-4	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
22-Jan-91	-3	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
29-Jan-91	-2	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
05-Feb-91	-1	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
12-Feb-91	0	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
19-Feb-91	+1	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
26-Feb-91	+2	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
05-Mar-91	+3	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
12-Mar-91	+4	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
19-Mar-91	+5	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
26-Mar-91	+6	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
02-Apr-91	+7	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
09-Apr-91	+8	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
16-Apr-91	+9	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
23-Apr-91	+10	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
30-Apr-91	+11	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
07-May-91	+12	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
14-May-91	+13	0	0	0	0	0	1	0	0	0.2	0.2	0	0	0	0	0	0	0	0	0.0	0.0	-	-
21-May-91	+14	3	**	1	1	0	1	1	3	1.4	0.3	0	0	0	0	0	0	0	0	0.0	0.0	-	-
28-May-91	+15	4	**	4	4	1	**	1	7	3.4	0.8	0	0	0	0	0	0	0	0	0.0	0.0	-	-
04-Jun-91	+16	10	**	9	7	1	**	3	4	5.6	1.4	0	0	0	0	0	0	0	0	0.0	0.0	-	-
11-Jun-91	+17	27	**	13	26	4	**	5	14	15.0	3.7	0	0	0	0	0	0	0	0	0.0	0.0	-	-
18-Jun-91	+18	15	**	**	12	**	**	13	15	13.8	0.6	0	0	0	0	0	0	0	0	0.0	0.0	-	-
25-Jun-91	+19	14	**	**	14	**	**	17	10	13.8	1.2	0	0	0	0	0	0	0	0	0.0	0.0	-	-
02-Jul-91	+20	26	**	**	21	**	**	39	35	30.3	3.6	0	0	0	0	0	0	0	0	0.0	0.0	-	-
09-Jul-91	+21	107	**	**	29	**	**	79	27	60.5	17.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
16-Jul-91	+22	13	**	**	9	**	**	20	35	19.2	5.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
23-Jul-91	+23	55	**	**	34	**	**	20	52	40.0	7.1	0	0	0	0	0	0	0	0	0.0	0.0	-	-
30-Jul-91	+24	82	**	**	124	**	**	208	504	229.5	82.6	0	0	0	0	0	0	0	0	0.0	0.0	-	-
06-Aug-91	+25	35	**	**	237	**	**	46	22	84.8	44.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
13-Aug-91	+26	98	**	**	60	**	**	**	46	67.7	12.6	0	0	0	0	0	0	0	0	0.0	0.0	-	-
20-Aug-91	+27	**	**	**	338	**	**	**	52	195.0	101.1	0	0	0	0	0	0	0	0	0.0	0.0	-	-
27-Aug-91	+28	**	**	**	245	**	**	**	122	183.5	43.5	0	0	0	0	0	0	0	0	0.0	0.0	-	-

Statistics = results of Mann-Whitney U test; U = U statistic; P = two-tailed P value (probability); - = analysis not done; ** = animals dead.

Appendix Table 8.4.4a
Some Carcass Data of the Buffalo Calves
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

	Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
Carcass wt (kg)	88	157	89	108	79	86	91	136	104.3	9.226	156	120	92	174	115	115	136	173	135.1	9.9	-	-
Dressed carcass (kg) ..	-	-	-	-	-	-	-	-	NA	NA	-	-	-	-	-	-	-	-	NA	NA	NA	NA
Weight of liver (kg)	2.50	3.51	2.60	2.20	2.00	2.13	1.75	2.40	2.386	0.18	2.00	1.30	1.10	1.90	1.40	1.60	1.50	1.75	1.569	0.10	3.0	0.0011
Weight of abdominal fat (kg)	-	-	-	-	-	-	-	-	NA	NA	-	-	-	-	-	-	-	-	NA	NA	NA	NA
Killing out %	-	-	-	-	-	-	-	-	NA	NA	-	-	-	-	-	-	-	-	NA	NA	NA	NA
Ratio of liver wt. to body wt.	0.03	0.22	0.03	0.02	0.03	0.03	0.02	0.02	0.048	0.023	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.012	0.00	0.0	0.0002

Statistics = results of Mann-Whitney U test; U = U statistic; - = Estimation not done; NA = not applicable.

Appendix Table 8.4.5
RBC Counts (x1012 cells/litre of blood) of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	5.19	7.31	6.44	6.58	5.42	5.50	6.60	6.40	6.18	0.24	5.00	6.67	5.08	5.82	6.53	7.07	5.86	7.20	6.15	0.28	-	-
07-Jan-91	-5	5.96	8.07	7.01	7.26	5.60	5.44	6.20	6.60	6.52	0.30	7.40	5.50	4.10	6.08	6.13	6.71	6.12	7.80	6.23	0.38	-	-
14-Jan-91	-4	5.80	8.20	6.80	7.10	6.49	6.45	6.98	6.17	6.75	0.24	7.41	7.50	5.10	6.87	7.23	8.32	6.01	7.42	6.98	0.33	22.0	0.3282
21-Jan-91	-3	6.20	7.55	7.56	6.77	6.01	6.11	6.24	7.00	6.68	0.21	8.14	7.64	5.76	6.51	7.37	7.80	5.64	8.00	7.11	0.33	-	-
28-Jan-91	-2	6.28	7.74	7.91	6.95	6.36	6.07	5.06	6.38	6.59	0.31	8.92	5.45	6.52	6.97	6.98	7.28	6.29	5.52	6.74	0.37	-	-
04-Feb-91	-1	6.20	7.86	7.00	7.78	5.58	5.60	5.57	6.81	6.55	0.32	9.69	6.31	6.00	7.04	6.57	7.63	5.11	7.66	7.00	0.46	-	-
11-Feb-91	0	6.24	7.19	7.75	7.62	5.39	4.90	6.93	5.92	6.49	0.35	8.16	6.59	6.36	6.08	7.80	5.20	6.08	9.84	7.01	0.49	25.0	0.5054
18-Feb-91	+1	6.80	7.96	7.53	7.37	6.36	5.31	5.60	6.22	6.64	0.31	7.65	5.82	7.57	7.05	5.64	6.71	5.52	5.75	6.46	0.29	-	-
25-Feb-91	+2	6.85	7.80	7.04	8.68	6.62	4.87	4.87	5.78	6.56	0.44	8.60	5.50	7.10	7.05	6.02	7.48	4.59	5.29	6.45	0.44	-	-
04-Mar-91	+3	6.10	7.10	6.00	7.30	7.12	5.26	6.63	6.24	6.47	0.23	7.28	5.88	6.02	7.63	6.01	6.61	5.72	6.10	6.41	0.23	-	-
11-Mar-91	+4	5.05	7.88	6.21	7.26	6.36	4.36	5.84	5.10	6.01	0.39	7.80	6.10	6.28	7.67	5.44	7.05	5.94	6.59	6.61	0.28	22.0	0.3282
18-Mar-91	+5	5.31	7.24	6.50	6.39	5.52	4.39	5.04	5.76	5.77	0.30	6.86	5.15	5.77	6.65	5.47	7.25	5.02	5.78	5.99	0.27	-	-
25-Mar-91	+6	6.05	7.66	6.47	5.74	5.07	4.42	5.06	5.50	5.75	0.33	7.76	6.06	6.03	6.33	5.03	6.03	5.63	6.06	6.12	0.26	-	-
01-Apr-91	+7	5.56	8.40	6.00	5.59	4.66	4.06	5.00	5.72	5.62	0.43	7.15	6.56	5.03	6.11	5.54	6.03	5.39	6.54	6.04	0.23	-	-
08-Apr-91	+8	5.39	7.06	6.89	5.61	4.80	5.28	5.10	5.49	5.70	0.27	6.60	5.67	5.05	6.53	7.46	6.86	5.12	6.40	6.21	0.28	23.0	0.3823
15-Apr-91	+9	5.01	8.08	5.64	7.02	3.70	4.25	5.31	6.31	5.67	0.48	6.85	5.86	5.65	7.44	5.36	6.10	4.46	6.57	6.04	0.31	-	-
22-Apr-91	+10	6.75	6.27	6.00	5.67	4.60	4.71	5.12	4.24	5.42	0.29	6.73	5.25	5.42	6.85	4.84	6.13	5.72	7.00	5.99	0.27	-	-
29-Apr-91	+11	5.10	6.08	5.33	7.25	4.89	4.01	3.80	4.22	5.09	0.38	6.90	6.53	5.83	6.33	4.87	7.21	4.51	6.78	6.12	0.32	-	-
06-May-91	+12	6.75	5.63	4.27	5.80	3.47	3.30	4.32	6.25	4.97	0.43	7.10	5.46	6.17	6.78	5.75	6.50	5.01	5.64	6.05	0.24	17.0	0.1304
13-May-91	+13	4.50	4.81	3.11	5.90	2.63	3.05	3.83	4.04	3.98	0.36	6.60	5.80	4.88	4.97	5.25	6.27	4.56	4.71	5.38	0.25	8.0	0.0104
20-May-91	+14	3.82	1.52	3.17	4.09	2.80	2.67	3.84	5.53	3.43	0.39	6.40	5.48	5.19	6.55	5.84	6.02	4.80	5.92	5.78	0.20	3.0	0.0011
27-May-91	+15	4.16	**	2.45	5.20	2.45	2.65	5.01	4.33	3.75	0.42	7.05	5.68	5.80	7.46	6.28	6.58	5.40	6.41	6.33	0.23	0.0	0.0003
03-Jun-91	+16	3.76	**	3.72	4.81	3.23	**	3.76	4.68	3.99	0.23	6.46	6.30	6.30	7.00	6.56	8.20	4.85	7.88	6.69	0.34	0.0	0.0007
10-Jun-91	+17	5.12	**	2.38	3.92	1.67	**	4.57	4.50	3.69	0.51	6.95	5.73	7.50	6.22	5.95	5.87	5.42	5.25	6.11	0.25	-	-
17-Jun-91	+18	3.36	**	1.80	4.42	1.60	**	3.62	4.63	3.24	0.48	8.03	6.32	6.93	6.52	6.46	6.93	5.97	6.76	6.74	0.20	-	-
24-Jun-91	+19	2.95	**	**	3.45	**	**	2.63	3.27	3.08	0.16	6.53	5.75	5.32	6.03	5.66	5.68	5.40	5.58	5.74	0.13	-	-
01-Jul-91	+20	2.97	**	**	4.10	**	**	2.93	3.45	3.36	0.24	7.53	6.62	6.10	7.85	6.03	7.12	6.30	6.37	6.74	0.23	0.0	0.0040
08-Jul-91	+21	2.83	**	**	3.28	**	**	3.05	3.98	3.29	0.22	7.20	6.05	5.93	6.65	6.37	6.65	5.53	6.18	6.32	0.17	-	-
15-Jul-91	+22	3.18	**	**	3.72	**	**	2.62	3.62	3.29	0.22	7.36	6.25	5.42	5.78	5.30	5.30	4.97	6.50	5.86	0.26	-	-
22-Jul-91	+23	3.48	**	**	3.17	**	**	2.17	3.57	3.10	0.28	6.65	5.83	5.38	5.35	5.28	5.88	5.13	5.93	5.68	0.16	-	-
29-Jul-91	+24	2.57	**	**	3.38	**	**	2.62	3.97	3.14	0.29	8.53	6.53	6.88	6.95	7.33	6.97	6.22	5.93	6.92	0.26	0.0	0.0040
05-Aug-91	+25	2.15	**	**	3.42	**	**	2.18	3.77	2.88	0.36	6.83	5.87	6.42	5.52	6.98	6.77	5.62	6.00	6.25	0.19	-	-
12-Aug-91	+26	2.08	**	**	2.78	**	**	2.30	3.68	2.71	0.31	7.40	5.98	6.63	6.05	6.28	6.85	6.17	6.62	6.50	0.16	-	-
19-Aug-91	+27	**	**	**	2.70	**	**	**	3.08	2.89	0.13	7.33	5.87	6.72	5.95	6.17	6.92	6.27	6.60	6.48	0.17	-	-
26-Aug-91	+28	**	**	**	2.55	**	**	**	3.15	2.85	0.21	6.58	5.60	5.62	6.25	5.88	6.50	6.35	6.82	6.20	0.15	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead;

the weeks when the median values of infected and uninfected groups were significantly different are printed in bold faces.

Appendix Table 8.4.6
Weekly Packed Cell Volumes (l/l) of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	0.32	0.37	0.36	0.36	0.32	0.30	0.31	0.32	0.33	0.01	0.35	0.34	0.29	0.31	0.34	0.34	0.33	0.32	0.33	0.01	-	-
07-Jan-91	-5	0.35	0.38	0.35	0.37	0.32	0.34	0.32	0.33	0.35	0.01	0.32	0.40	0.22	0.32	0.33	0.40	0.30	0.33	0.33	0.02	-	-
14-Jan-91	-4	0.32	0.36	0.35	0.37	0.31	0.32	0.33	0.32	0.34	0.01	0.38	0.36	0.27	0.33	0.32	0.41	0.32	0.35	0.34	0.01	26.5	0.5737
21-Jan-91	-3	0.32	0.35	0.37	0.37	0.32	0.31	0.33	0.33	0.34	0.01	0.36	0.41	0.28	0.33	0.33	0.42	0.29	0.32	0.34	0.02	-	-
28-Jan-91	-2	0.32	0.36	0.36	0.36	0.33	0.30	0.35	0.32	0.34	0.01	0.39	0.37	0.30	0.33	0.30	0.39	0.29	0.33	0.34	0.01	-	-
04-Feb-91	-1	0.34	0.36	0.32	0.36	0.34	0.31	0.30	0.31	0.33	0.01	0.32	0.32	0.31	0.33	0.29	0.39	0.29	0.32	0.32	0.01	-	-
11-Feb-91	0	0.33	0.36	0.34	0.35	0.35	0.28	0.31	0.29	0.33	0.01	0.31	0.33	0.33	0.34	0.30	0.36	0.33	0.33	0.33	0.01	30.5	0.8785
18-Feb-91	+1	0.34	0.33	0.33	0.32	0.34	0.28	0.29	0.32	0.32	0.01	0.35	0.32	0.32	0.36	0.27	0.34	0.30	0.30	0.32	0.01	-	-
25-Feb-91	+2	0.30	0.34	0.31	0.33	0.32	0.27	0.28	0.29	0.31	0.01	0.36	0.31	0.31	0.34	0.28	0.35	0.29	0.27	0.31	0.01	-	-
04-Mar-91	+3	0.28	0.33	0.28	0.31	0.32	0.26	0.27	0.30	0.29	0.01	0.35	0.29	0.30	0.31	0.28	0.32	0.28	0.27	0.30	0.01	-	-
11-Mar-91	+4	0.29	0.32	0.29	0.33	0.30	0.23	0.25	0.27	0.29	0.01	0.35	0.31	0.32	0.31	0.27	0.32	0.27	0.30	0.31	0.01	20.5	0.2345
18-Mar-91	+5	0.28	0.32	0.31	0.30	0.28	0.24	0.26	0.27	0.28	0.01	0.35	0.28	0.30	0.31	0.27	0.33	0.29	0.27	0.30	0.01	-	-
25-Mar-91	+6	0.28	0.33	0.29	0.28	0.27	0.24	0.25	0.28	0.28	0.01	0.35	0.30	0.27	0.30	0.26	0.31	0.26	0.30	0.29	0.01	-	-
01-Apr-91	+7	0.30	0.32	0.29	0.28	0.28	0.25	0.27	0.29	0.29	0.01	0.36	0.30	0.28	0.30	0.30	0.32	0.29	0.31	0.31	0.01	-	-
08-Apr-91	+8	0.28	0.32	0.30	0.27	0.30	0.24	0.26	0.30	0.28	0.01	0.31	0.30	0.29	0.31	0.30	0.34	0.28	0.30	0.30	0.01	18.0	0.1605
15-Apr-91	+9	0.29	0.30	0.30	0.30	0.27	0.24	0.27	0.32	0.29	0.01	0.31	0.31	0.28	0.31	0.28	0.32	0.27	0.28	0.30	0.01	24.5	0.4418
22-Apr-91	+10	0.29	0.30	0.27	0.30	0.26	0.23	0.26	0.29	0.28	0.01	0.35	0.31	0.30	0.34	0.28	0.33	0.28	0.31	0.31	0.01	9.0	0.0148
29-Apr-91	+11	0.27	0.27	0.26	0.29	0.25	0.21	0.25	0.32	0.27	0.01	0.37	0.34	0.31	0.30	0.28	0.34	0.27	0.30	0.31	0.01	8.0	0.0104
06-May-91	+12	0.29	0.25	0.22	0.27	0.19	0.18	0.26	0.32	0.25	0.02	0.36	0.30	0.30	0.35	0.28	0.34	0.29	0.31	0.32	0.01	6.5	0.0047
13-May-91	+13	0.23	0.22	0.18	0.25	0.17	0.16	0.24	0.31	0.22	0.02	0.33	0.32	0.30	0.31	0.30	0.31	0.28	0.29	0.31	0.01	-	-
20-May-91	+14	0.18	0.06	0.15	0.25	0.15	0.15	0.22	0.29	0.18	0.02	0.32	0.32	0.31	0.32	0.28	0.33	0.28	0.30	0.31	0.01	-	-
27-May-91	+15	0.18	**	0.13	0.24	0.13	0.14	0.20	0.26	0.18	0.02	0.32	0.32	0.29	0.32	0.33	0.31	0.29	0.30	0.31	0.00	-	-
03-Jun-91	+16	0.18	**	0.13	0.27	0.15	**	0.19	0.25	0.20	0.02	0.32	0.32	0.31	0.31	0.30	0.36	0.30	0.31	0.32	0.01	0.0	0.0007
10-Jun-91	+17	0.17	**	0.12	0.22	0.13	**	0.18	0.19	0.17	0.01	0.34	0.33	0.30	0.35	0.31	0.34	0.31	0.30	0.32	0.01	-	-
17-Jun-91	+18	0.18	**	0.10	0.22	0.11	**	0.17	0.22	0.17	0.02	0.36	0.33	0.31	0.31	0.30	0.34	0.28	0.30	0.32	0.01	-	-
24-Jun-91	+19	0.16	**	**	0.21	**	**	0.18	0.21	0.19	0.01	0.33	0.33	0.32	0.33	0.30	0.34	0.32	0.31	0.32	0.00	-	-
01-Jul-91	+20	0.14	**	**	0.18	**	**	0.18	0.22	0.18	0.01	0.33	0.34	0.31	0.35	0.30	0.31	0.30	0.31	0.32	0.01	0.0	0.0040
08-Jul-91	+21	0.15	**	**	0.18	**	**	0.18	0.25	0.19	0.02	0.35	0.34	0.32	0.35	0.30	0.36	0.30	0.32	0.33	0.01	-	-
15-Jul-91	+22	0.16	**	**	0.17	**	**	0.18	0.24	0.19	0.02	0.35	0.30	0.30	0.35	0.30	0.34	0.33	0.33	0.33	0.01	-	-
22-Jul-91	+23	0.17	**	**	0.18	**	**	0.19	0.24	0.20	0.01	0.32	0.30	0.31	0.31	0.30	0.35	0.30	0.30	0.31	0.01	-	-
29-Jul-91	+24	0.14	**	**	0.19	**	**	0.16	0.25	0.19	0.02	0.36	0.34	0.32	0.34	0.32	0.37	0.30	0.34	0.34	0.01	0.0	0.0040
05-Aug-91	+25	0.15	**	**	0.16	**	**	0.15	0.23	0.17	0.02	0.35	0.30	0.33	0.32	0.30	0.37	0.31	0.30	0.32	0.01	-	-
12-Aug-91	+26	0.12	**	**	0.16	**	**	0.13	0.24	0.16	0.02	0.35	0.33	0.33	0.34	0.30	0.35	0.33	0.34	0.33	0.01	-	-
19-Aug-91	+27	**	**	**	0.15	**	**	**	0.16	0.16	0.00	0.35	0.31	0.32	0.30	0.30	0.35	0.31	0.32	0.32	0.01	-	-
26-Aug-91	+28	**	**	**	0.13	**	**	**	0.20	0.17	0.02	0.32	0.29	0.31	0.33	0.30	0.34	0.29	0.30	0.31	0.01	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead;

the weeks when the median values of infected and uninfected groups were significantly different are printed in bold faces.

Appendix Table 8.4.7
Haemoglobin Concentrations (g/l of blood) of the Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	128	144	137	138	123	124	135	140	133.6	2.6	136	128	110	122	113	138	119	135	125.1	3.6	-	-
07-Jan-91	-5	121	139	131	131	127	125	132	128	129.2	1.7	135	134	112	121	118	125	121	122	123.4	2.6	-	-
14-Jan-91	-4	125	135	126	137	120	126	135	127	128.7	2.0	142	136	102	138	124	150	122	124	129.5	4.9	30.0	0.8785
21-Jan-91	-3	125	142	133	132	126	130	112	142	130.4	3.2	141	147	116	130	134	143	115	124	131.0	4.0	-	-
28-Jan-91	-2	125	151	136	147	124	122	113	125	130.4	4.3	142	148	128	133	118	149	122	144	135.6	3.9	-	-
04-Feb-91	-1	131	143	129	129	123	112	123	121	126.2	3.0	135	119	121	124	111	145	113	123	123.9	3.7	-	-
11-Feb-91	0	136	132	118	133	133	109	109	122	124.0	3.7	135	117	129	134	113	134	116	115	124.1	3.2	31.0	0.9591
18-Feb-91	+1	123	121	116	129	127	111	102	116	118.0	2.9	122	116	119	139	103	119	119	120	119.4	3.3	-	-
25-Feb-91	+2	110	134	114	130	117	110	117	106	117.3	3.3	134	112	122	138	125	128	120	113	123.9	3.1	-	-
04-Mar-91	+3	114	132	110	128	136	101	114	130	120.6	4.1	132	123	117	125	116	129	125	114	122.4	2.1	-	-
11-Mar-91	+4	104	120	105	108	106	99	102	116	107.3	2.3	112	109	109	112	106	116	113	119	112.1	1.4	16.0	0.1049
18-Mar-91	+5	106	122	111	100	106	95	104	116	107.5	2.8	129	100	114	129	100	116	114	116	114.8	3.6	-	-
25-Mar-91	+6	105	128	105	100	104	90	105	118	107.0	3.8	120	113	118	113	120	105	113	120	115.4	1.8	-	-
01-Apr-91	+7	111	129	111	111	115	100	107	113	112.3	2.7	127	129	111	127	123	127	115	115	121.8	2.3	-	-
08-Apr-91	+8	95	122	103	104	118	99	103	114	107.3	3.2	114	114	108	126	114	149	114	109	118.7	4.4	16.0	0.1049
15-Apr-91	+9	116	115	108	108	112	104	102	116	110.2	1.8	112	128	108	124	114	113	114	113	115.8	2.2	20.5	0.2345
22-Apr-91	+10	102	124	113	109	97	90	109	124	108.5	4.0	129	124	113	124	109	119	113	113	118.0	2.3	15.5	0.0830
29-Apr-91	+11	109	128	139	128	94	75	113	113	112.5	6.8	158	132	132	124	113	128	113	117	127.0	4.9	18.0	0.1605
06-May-91	**+12**	117	105	87	124	79	73	93	109	98.4	6.1	139	128	117	139	117	143	128	135	130.8	3.4	3.0	0.0011
13-May-91	**+13**	75	75	49	114	49	53	90	110	77.0	8.7	123	123	115	131	113	120	128	130	122.9	2.2	-	-
20-May-91	**+14**	73	26	62	103	59	55	81	117	72.0	9.5	121	125	147	147	110	125	128	103	125.6	5.1	-	-
27-May-91	**+15**	68	**	45	90	53	37	75	94	66.0	7.7	128	124	128	139	132	122	120	120	126.7	2.1	-	-
03-Jun-91	**+16**	70	**	40	77	45	**	65	92	65.0	7.3	124	120	124	129	100	131	131	128	123.3	3.3	0.0	0.0007
10-Jun-91	**+17**	40	**	31	80	40	**	78	94	60.3	9.8	129	119	124	121	103	121	131	121	121.4	2.8	-	-
17-Jun-91	**+18**	36	**	28	77	33	**	77	97	57.9	10.9	127	121	128	121	116	135	129	124	125.2	1.9	-	-
24-Jun-91	**+19**	39	**	**	70	**	**	70	85	65.7	8.4	116	124	116	116	116	131	116	116	118.8	1.9	-	-
01-Jul-91	**+20**	54	**	**	62	**	**	62	77	63.8	4.2	116	131	101	124	116	131	116	124	119.8	3.3	0.0	0.0040
08-Jul-91	**+21**	46	**	**	54	**	**	62	77	59.9	5.7	124	108	116	131	104	116	104	116	115.0	3.1	-	-
15-Jul-91	**+22**	39	**	**	58	**	**	66	75	59.3	6.7	119	105	105	116	101	131	116	128	115.0	3.7	-	-
22-Jul-91	**+23**	62	**	**	66	**	**	77	85	72.5	4.6	116	116	124	131	116	133	113	116	120.6	2.5	-	-
29-Jul-91	**+24**	50	**	**	70	**	**	54	93	66.7	8.4	139	116	116	124	131	116	116	120	122.2	2.9	0.0	0.0040
05-Aug-91	**+25**	46	**	**	58	**	**	52	77	58.4	5.8	147	131	146	131	108	135	124	108	128.9	4.9	-	-
12-Aug-91	+26	30	**	**	43	**	**	35	85	48.3	10.9	130	113	113	130	113	130	130	121	122.4	2.8	-	-
19-Aug-91	+27	**	**	**	46	**	**	**	58	52.2	4.1	126	101	119	112	112	116	114	118	114.6	2.4	-	-
26-Aug-91	+28	**	**	**	31	**	**	**	54	42.5	8.2	116	93	116	108	131	116	101	108	111.1	3.8	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead;

the weeks when the median values of infected and uninfected groups were significantly different are printed in bold faces.

Appendix Table 8.4.8
Mean Cell Volumes (fl) of the Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	61.7	50.6	55.9	54.7	59.0	54.5	47.0	50.0	54.2	1.61	70.0	51.0	57.1	53.3	52.1	48.1	56.3	44.4	54.0	2.53	-	-
07-Jan-91	-5	58.7	47.1	49.9	51.0	57.1	62.5	51.6	50.0	53.5	1.76	43.2	72.7	53.7	52.6	53.8	59.6	49.0	42.3	53.4	3.21	-	-
14-Jan-91	-4	55.2	43.9	51.5	52.1	47.8	49.6	47.3	51.9	49.9	1.16	51.3	48.0	52.9	48.0	44.3	49.3	53.2	47.2	49.3	1.02	26.1	0.0585
21-Jan-91	-3	51.6	46.4	48.9	54.7	53.2	50.7	52.9	47.1	50.7	0.99	44.2	53.7	48.6	50.7	44.8	53.8	51.4	40.0	48.4	1.64	-	-
28-Jan-91	-2	51.0	46.5	45.5	51.8	51.9	49.4	69.2	50.2	51.9	2.43	43.7	67.9	46.0	47.3	43.0	53.6	46.1	59.8	50.9	2.93	-	-
04-Feb-91	-1	54.8	45.8	45.7	46.3	60.9	55.4	53.9	45.5	51.0	1.97	33.0	50.7	51.7	46.9	44.1	51.1	56.8	41.8	47.0	2.43	-	-
11-Feb-91	0	52.9	50.1	43.9	45.9	64.9	57.1	44.7	49.0	51.1	2.36	38.0	50.1	51.9	55.9	38.5	69.2	54.3	33.5	48.9	3.88	29.5	0.7984
18-Feb-91	+1	50.0	41.5	43.8	43.4	53.5	52.7	51.8	51.4	48.5	1.59	45.8	55.0	42.3	51.1	47.9	50.7	54.3	52.2	49.9	1.44	-	-
25-Feb-91	+2	43.8	43.6	44.0	45.2	48.3	55.4	57.5	50.2	48.5	1.81	41.9	56.4	43.7	48.2	46.5	46.8	63.2	51.0	49.7	2.33	-	-
04-Mar-91	+3	45.9	46.5	46.7	42.5	44.9	49.4	40.7	48.1	45.6	0.94	48.1	49.3	49.8	40.6	46.6	48.4	49.0	44.3	47.0	1.04	-	-
11-Mar-91	+4	57.4	40.6	46.7	45.5	47.2	52.8	42.8	52.9	48.2	1.88	47.9	43.7	44.0	44.3	49.6	50.0	45.5	48.4	46.7	0.87	28.5	0.7209
18-Mar-91	+5	52.7	44.2	47.7	46.9	50.7	54.7	51.6	46.9	49.4	1.17	44.9	38.9	44.1	46.6	49.4	45.5	57.8	43.5	46.3	1.82	-	-
25-Mar-91	+6	46.3	43.1	44.8	48.8	53.3	54.3	49.4	50.9	48.9	1.31	45.1	49.5	44.8	47.4	51.7	51.4	46.2	49.5	48.2	0.90	-	-
01-Apr-91	+7	54.6	43.2	48.3	50.1	60.1	61.6	54.0	50.7	52.7	2.00	50.3	45.7	55.7	49.1	54.2	53.1	53.8	47.4	51.2	1.18	-	-
08-Apr-91	+8	51.9	45.3	43.5	48.1	62.5	45.5	51.0	54.6	50.3	2.05	47.0	52.9	57.4	47.5	40.2	49.6	54.7	46.9	49.5	1.79	31.0	0.9591
15-Apr-91	+9	57.9	41.7	53.2	42.7	73.0	56.5	50.8	50.7	53.3	3.25	45.3	52.9	49.6	41.7	52.2	52.5	49.1	42.6	48.2	1.49	-	-
22-Apr-91	+10	43.0	42.9	45.0	52.9	56.5	48.8	50.8	68.4	51.0	2.82	52.0	59.0	55.4	49.6	57.9	53.8	49.0	44.3	52.6	1.63	-	-
29-Apr-91	+11	52.9	44.4	48.8	40.0	51.1	52.4	65.8	75.8	53.9	3.84	53.6	52.1	48.0	47.4	57.5	33.3	49.1	44.2	48.2	2.41	-	-
06-May-91	+12	60.4	44.4	51.5	46.6	54.8	54.5	66.7	71.1	56.2	3.08	50.7	54.9	54.5	51.6	48.7	52.3	57.9	55.0	53.2	0.97	28.5	0.7209
13-May-91	+13	51.1	45.7	57.9	42.4	64.6	52.5	62.7	62.0	54.9	2.73	50.0	55.2	50.8	47.7	50.8	49.4	50.0	50.9	50.6	0.70	-	-
20-May-91	+14	47.1	39.5	47.3	61.1	53.6	56.2	57.3	52.4	51.8	2.28	50.0	58.4	59.7	48.9	43.1	54.8	48.3	43.5	50.8	2.08	-	-
27-May-91	+15	43.3	**	53.1	46.2	53.1	52.8	39.9	60.0	49.8	2.43	45.4	56.3	50.0	42.9	52.5	47.1	53.7	46.8	49.3	1.52	-	-
03-Jun-91	+16	47.9	**	48.1	47.8	68.2	**	50.5	53.4	52.7	2.95	49.5	50.8	49.2	44.3	45.7	43.9	50.8	39.3	46.7	1.35	14.0	0.2284
10-Jun-91	+17	48.6	**	50.4	56.1	77.8	**	39.4	42.2	52.4	5.14	48.9	49.3	40.0	56.3	52.1	43.0	48.4	44.1	47.8	1.73	-	-
17-Jun-91	+18	53.6	**	55.6	49.8	68.8	**	47.0	47.5	53.7	3.02	44.8	52.2	44.7	47.5	46.4	49.1	46.9	44.4	47.0	0.87	-	-
24-Jun-91	+19	54.2	**	**	60.9	**	**	68.4	64.2	61.9	2.60	44.0	48.5	50.8	54.7	44.8	50.7	50.0	47.0	48.8	1.17	-	-
01-Jul-91	+20	47.1	**	**	43.9	**	**	61.4	63.8	54.1	4.33	43.8	51.4	50.8	44.6	49.8	43.5	47.6	48.7	47.5	1.05	11.0	0.4606
08-Jul-91	**+21**	53.6	**	**	54.9	**	**	59.0	62.8	57.4	1.90	48.6	56.2	46.4	52.6	47.1	54.1	46.2	51.8	50.4	1.27	3.0	0.0283
15-Jul-91	**+22**	44.7	**	**	45.7	**	**	68.7	66.3	56.3	5.60	47.5	48.0	46.9	51.5	47.6	54.0	49.3	50.8	49.4	0.81	0.0	0.0040
22-Jul-91	**+23**	48.9	**	**	56.8	**	**	87.6	67.2	65.1	7.25	49.2	44.1	48.4	48.4	47.6	50.7	49.2	50.6	48.5	0.69	0.0	0.0040
29-Jul-91	**+24**	54.5	**	**	56.2	**	**	61.1	63.0	58.7	1.73	50.0	52.1	50.0	50.0	47.1	53.1	48.2	52.3	50.3	0.68	0.0	0.0040
05-Aug-91	**+25**	69.5	**	**	46.8	**	**	68.8	61.0	61.6	4.60	55.3	51.1	51.4	49.1	43.0	54.7	55.2	50.0	51.2	1.36	0.0	0.0040
12-Aug-91	**+26**	57.7	**	**	57.6	**	**	56.5	65.2	59.2	1.74	47.3	55.2	49.8	56.2	47.8	51.1	53.5	51.4	51.5	1.08	0.0	0.0040
19-Aug-91	+27	**	**	**	55.6	**	**	**	51.9	53.8	1.28	47.7	54.7	47.6	50.4	48.6	50.6	49.4	48.5	49.7	0.76	-	-
26-Aug-91	+28	**	**	**	51.0	**	**	**	57.1	54.1	2.18	48.6	51.8	55.2	52.8	51.0	52.3	45.7	44.0	50.2	1.25	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead;

the weeks when the median values of infected and uninfected groups were significantly different are printed in bold faces.

Appendix Table 8.4.9
Mean Cell Haemoglobin (pg) of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	24.6	19.7	21.2	21.0	22.7	22.5	20.5	21.9	21.76	0.50	27.2	19.3	21.6	20.9	17.4	19.5	20.2	18.8	20.60	0.98	-	-
07-Jan-91	-5	20.3	17.2	18.6	18.1	22.6	23.0	21.3	19.4	20.06	0.70	18.2	24.4	27.4	19.9	19.2	18.6	19.8	15.6	20.38	1.23	-	-
14-Jan-91	-4	21.6	16.5	18.5	19.3	18.4	19.5	19.3	20.5	19.20	0.50	19.1	18.1	20.1	20.0	17.1	18.0	20.3	16.6	18.66	0.47	25.0	0.5054
21-Jan-91	-3	20.2	18.9	17.6	19.5	20.9	21.3	17.9	20.3	19.58	0.45	17.3	19.2	20.1	20.0	18.1	18.3	20.4	15.5	18.60	0.55	-	-
28-Jan-91	-2	19.9	19.5	17.2	21.1	19.5	20.1	22.3	19.6	19.92	0.49	15.9	27.2	19.7	19.1	17.0	20.5	19.4	26.0	20.59	1.32	-	-
04-Feb-91	-1	21.1	18.2	18.4	16.5	22.0	20.1	22.0	17.8	19.51	0.69	13.9	18.8	20.2	17.6	16.9	19.0	22.1	16.0	18.08	0.84	-	-
11-Feb-91	0	21.7	18.4	15.2	17.5	24.7	22.2	15.8	20.6	19.50	1.10	16.5	17.8	20.3	22.0	14.5	25.8	19.1	11.7	18.46	1.46	27.0	0.6454
18-Feb-91	+1	18.1	15.2	15.4	17.4	20.0	20.9	18.2	18.6	17.98	0.66	16.0	19.9	15.7	19.7	18.2	17.7	21.5	20.8	18.68	0.71	-	-
25-Feb-91	+2	16.1	17.2	16.1	17.8	17.7	22.6	24.1	18.3	18.74	0.98	15.6	20.4	17.1	19.5	20.7	17.2	26.0	21.3	19.73	1.08	-	-
04-Mar-91	+3	18.6	18.6	18.3	17.6	19.0	19.2	17.3	20.9	18.68	0.37	18.1	20.9	19.5	16.3	19.3	19.5	21.8	18.6	19.24	0.55	-	-
11-Mar-91	+4	20.6	14.6	16.8	14.1	16.6	21.2	17.4	22.6	17.99	1.05	15.4	15.4	15.0	16.1	19.5	18.1	19.1	19.2	17.21	0.65	28.0	0.7209
18-Mar-91	+5	20.0	16.8	17.0	15.5	19.2	21.2	19.6	18.4	18.47	0.63	16.5	13.9	16.8	19.4	18.3	18.7	22.8	18.7	18.14	0.85	-	-
25-Mar-91	+6	17.4	16.7	16.3	17.1	19.3	20.4	20.8	20.2	18.53	0.61	15.5	18.6	19.5	17.8	23.9	17.5	20.1	19.9	19.11	0.81	-	-
01-Apr-91	+7	20.0	17.4	18.5	19.9	24.7	24.7	21.5	19.8	20.81	0.88	17.7	19.6	22.1	20.8	22.2	21.1	21.4	17.6	20.32	0.60	-	-
08-Apr-91	+8	17.7	17.3	14.9	18.4	24.6	18.8	20.2	20.8	19.10	0.95	17.3	20.2	21.4	19.3	15.3	21.7	22.3	17.1	19.33	0.84	29.0	0.7984
15-Apr-91	+9	23.1	15.9	19.2	15.4	30.3	24.6	19.2	18.4	20.76	1.64	16.4	21.8	19.2	16.6	21.3	18.5	20.8	17.2	18.97	0.72	-	-
22-Apr-91	+10	15.1	17.7	18.8	19.2	18.8	19.2	21.3	29.3	19.93	1.38	22.1	23.7	20.8	18.1	22.5	22.7	19.7	16.1	20.73	0.85	-	-
29-Apr-91	+11	21.4	21.1	20.5	17.6	17.2	18.8	27.1	26.8	21.30	1.25	21.5	18.6	19.2	19.6	23.2	17.7	20.5	17.2	19.69	0.65	-	-
06-May-91	+12	24.5	18.7	20.3	21.4	22.7	22.1	23.8	24.3	22.22	0.67	19.6	23.4	21.2	20.5	20.3	20.5	25.5	22.2	21.66	0.65	25.5	0.5054
13-May-91	+13	16.7	15.7	15.7	19.3	18.6	17.3	23.6	22.0	18.62	0.96	18.6	21.2	19.6	20.2	19.1	19.2	22.8	22.8	20.44	0.55	-	-
20-May-91	+14	19.2	17.1	19.7	25.1	18.1	18.7	21.0	21.2	20.02	0.82	18.9	22.8	28.3	22.4	16.9	20.7	22.1	14.9	20.87	1.35	-	-
27-May-91	+15	16.3	**	18.4	17.4	21.5	13.8	15.0	21.7	17.74	1.06	18.1	21.9	22.1	18.7	21.0	18.5	22.3	18.8	20.16	0.59	-	-
03-Jun-91	+16	18.5	**	14.9	16.1	20.6	**	17.3	19.7	17.86	0.82	19.1	19.0	19.6	18.4	15.3	16.0	22.3	16.2	18.24	0.76	23.0	0.9497
10-Jun-91	+17	11.3	**	13.2	20.3	23.8	**	17.0	20.8	17.74	1.78	18.6	17.8	16.6	19.5	17.4	15.3	20.5	17.9	17.94	0.54	-	-
17-Jun-91	+18	10.6	**	15.4	17.5	20.3	**	21.4	20.9	17.67	1.54	15.8	19.2	18.4	18.6	17.9	19.5	21.6	18.3	18.68	0.54	-	-
24-Jun-91	+19	13.1	**	**	20.2	**	**	26.5	26.0	21.43	2.71	15.5	18.2	18.4	19.2	17.3	19.6	18.1	17.6	17.98	0.42	-	-
01-Jul-91	+20	18.2	**	**	15.1	**	**	21.1	22.4	19.20	1.41	15.4	19.8	16.5	15.8	19.2	18.5	18.4	19.4	17.87	0.58	13.0	0.6828
08-Jul-91	+21	16.4	**	**	16.5	**	**	20.3	19.4	18.14	0.86	17.2	17.9	16.8	19.8	16.4	17.4	16.1	18.8	17.53	0.41	-	-
15-Jul-91	+22	10.8	**	**	15.6	**	**	25.1	20.7	18.04	2.69	16.2	16.8	16.4	17.0	16.0	20.9	17.3	19.6	17.51	0.58	-	-
22-Jul-91	+23	17.8	**	**	20.7	**	**	25.6	23.8	21.98	1.50	17.8	17.0	19.3	20.5	18.4	19.2	18.6	19.5	18.81	0.36	-	-
29-Jul-91	+24	16.5	**	**	18.6	**	**	20.6	23.4	19.79	1.27	19.3	17.7	18.1	20.2	19.3	19.6	18.6	18.4	18.92	0.27	12.5	0.5697
05-Aug-91	+25	21.6	**	**	17.0	**	**	23.9	20.5	20.72	1.24	21.6	20.7	19.6	20.2	15.5	20.0	22.0	18.0	19.70	0.69	-	-
12-Aug-91	+26	14.5	**	**	15.6	**	**	15.1	23.1	17.07	1.75	17.6	18.8	17.0	21.5	17.9	19.0	21.1	18.3	18.90	0.53	-	-
19-Aug-91	+27	**	**	**	17.2	**	**	**	18.8	18.01	0.58	17.2	17.8	17.6	18.7	18.1	16.7	18.2	17.9	17.79	0.20	-	-
26-Aug-91	+28	**	**	**	12.1	**	**	**	17.2	14.65	1.79	17.6	16.6	20.6	18.9	22.3	17.8	17.4	17.3	18.58	0.65	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead.

Appendix Table 8.4.10
Mean Cell Haemoglobin Concentration (g/l) of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	399	389	379	384	385	413	435	438	402.7	7.7	388	378	378	392	333	406	359	422	382.1	9.1	-	-
07-Jan-91	-5	346	364	373	355	396	368	413	388	375.4	7.3	421	336	510	378	357	312	403	370	385.7	20.2	-	-
14-Jan-91	-4	391	375	359	371	386	393	409	396	384.9	5.2	373	377	379	417	386	366	381	353	378.8	6.1	23.5	0.3823
21-Jan-91	-3	392	407	359	357	393	419	339	430	387.2	10.7	390	358	413	394	405	339	397	388	385.4	8.2	-	-
28-Jan-91	-2	391	420	378	408	375	407	323	392	386.7	9.9	364	400	428	403	395	382	421	435	403.5	7.9	-	-
04-Feb-91	-1	386	398	402	357	361	362	409	391	383.0	6.7	422	372	391	376	382	372	390	383	386.0	5.4	-	-
11-Feb-91	0	411	368	347	380	380	388	352	420	380.8	8.5	434	356	392	393	377	373	351	348	378.0	9.5	30.0	0.8785
18-Feb-91	+1	362	366	350	402	373	397	352	362	370.6	6.4	350	361	371	386	380	349	396	398	373.9	6.5	-	-
25-Feb-91	+2	367	395	366	393	367	407	419	366	385.0	7.1	373	362	392	405	445	367	412	417	396.6	9.5	-	-
04-Mar-91	+3	406	400	393	414	423	388	424	434	410.2	5.4	377	423	391	402	414	402	445	421	409.3	6.9	-	-
11-Mar-91	+4	359	359	360	309	352	403	406	428	371.9	12.5	320	352	341	363	393	362	420	398	368.4	10.9	30.5	0.8785
18-Mar-91	+5	379	380	357	330	379	388	381	393	373.3	6.7	368	358	381	415	371	412	394	430	391.1	8.6	-	-
25-Mar-91	+6	375	388	363	351	362	376	422	398	379.3	7.5	344	376	436	376	463	340	434	401	396.5	14.9	-	-
01-Apr-91	+7	370	403	383	397	411	401	397	390	394.2	4.2	352	430	397	424	410	397	397	372	397.4	8.5	-	-
08-Apr-91	+8	340	381	343	383	394	413	396	381	379.1	8.4	369	381	373	406	381	437	409	364	390.1	8.3	30.0	0.8785
15-Apr-91	+9	400	383	361	361	415	435	378	362	386.7	9.1	361	412	386	399	409	353	424	403	393.5	8.2	-	-
22-Apr-91	+10	350	414	418	364	333	393	420	428	390.0	12.0	425	401	376	365	390	422	403	364	393.2	7.8	-	-
29-Apr-91	+11	404	475	420	441	336	359	412	353	399.9	15.7	400	358	399	414	403	533	418	389	414.3	17.0	-	-
06-May-91	+12	405	422	394	460	415	405	357	341	399.8	12.3	387	426	389	398	417	391	441	405	406.7	6.5	31.0	0.9591
13-May-91	**+13**	327	342	272	456	288	329	376	355	343.3	18.8	372	384	385	424	376	388	457	447	404.2	11.1	8.5	0.0104
20-May-91	**+14**	307	433	315	386	338	333	367	345	353.0	13.7	378	390	473	458	393	378	458	342	408.8	15.8	10.0	0.0207
27-May-91	**+15**	376	**	348	376	405	262	377	362	358.0	16.0	400	388	441	435	399	393	415	401	409.1	6.5	5.0	0.0059
03-Jun-91	**+16**	387	**	308	336	303	**	343	370	341.1	12.3	386	374	399	415	335	365	438	411	390.4	10.7	8.0	0.0426
10-Jun-91	**+17**	234	**	262	362	305	**	432	493	347.8	37.4	380	362	414	346	333	356	424	405	377.5	11.1	-	-
17-Jun-91	**+18**	198	**	277	351	295	**	455	439	336.0	36.9	353	368	411	392	387	398	461	412	397.6	10.8	-	-
24-Jun-91	**+19**	242	**	**	331	**	**	387	405	341.1	31.8	351	375	362	351	386	386	362	374	368.5	4.7	-	-
01-Jul-91	**+20**	286	**	**	333	**	**	343	351	328.6	12.6	351	386	324	353	386	424	386	399	376.4	10.4	3.5	0.0283
08-Jul-91	**+21**	309	**	**	301	**	**	343	309	315.6	8.2	353	318	362	375	348	322	348	362	348.7	6.5	-	-
15-Jul-91	**+22**	241	**	**	341	**	**	365	313	314.9	23.2	341	350	350	331	335	386	351	386	353.8	7.1	-	-
22-Jul-91	**+23**	294	**	**	365	**	**	357	354	342.4	14.2	362	386	399	424	386	379	378	386	387.7	5.9	-	-
29-Jul-91	**+24**	259	**	**	336	**	**	338	331	316.1	16.6	386	381	362	364	411	313	386	352	369.5	9.6	3.0	0.0283
05-Aug-91	+25	309	**	**	363	**	**	347	336	338.6	9.7	391	405	382	411	361	366	399	361	384.3	6.7	-	-
12-Aug-91	+26	251	**	**	271	**	**	268	354	285.8	20.1	371	342	342	382	376	371	394	357	366.8	6.2	-	-
19-Aug-91	+27	**	**	**	309	**	**	**	363	335.9	18.8	360	325	370	372	372	331	369	369	358.5	6.3	-	-
26-Aug-91	+28	**	**	**	238	**	**	**	301	269.1	22.2	362	320	374	358	438	341	381	394	371.0	11.8	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead;

the weeks when the median values of infected and uninfected groups were significantly different are printed in bold faces.

Appendix Table 10.4.11
Total WBC Counts (x10⁹ cells/litre of blood) of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	9.7	9.0	8.1	10.8	10.3	6.5	9.3	8.8	9.04	0.44	7.1	10.4	5.2	9.6	7.8	6.4	9.5	10.1	8.24	0.63	-	-
07-Jan-91	-5	7.2	10.4	6.0	12.5	9.2	7.5	9.2	7.8	8.71	0.68	10.9	10.1	8.9	10.0	8.5	6.6	9.6	9.6	9.26	0.43	-	-
14-Jan-91	-4	10.5	13.0	8.6	13.6	7.3	7.7	7.1	6.9	9.33	0.90	11.4	9.5	8.9	9.4	5.6	6.1	8.5	9.8	8.65	0.64	30.0	0.8785
21-Jan-91	-3	11.6	8.3	8.5	12.4	9.5	6.2	10.5	6.6	9.19	0.74	11.8	11.8	5.6	11.1	8.0	7.4	10.5	8.1	9.26	0.76	-	-
28-Jan-91	-2	11.0	12.1	9.2	13.6	11.1	8.3	11.0	8.5	10.58	0.61	11.2	11.6	9.7	11.1	7.2	7.8	10.0	8.4	9.61	0.55	-	-
04-Feb-91	-1	12.1	9.6	7.9	9.4	10.7	7.5	11.9	9.0	9.76	0.56	12.5	9.1	9.4	12.6	7.1	7.6	9.4	11.5	9.90	0.70	-	-
11-Feb-91	0	12.2	11.6	6.7	11.9	9.0	7.0	12.0	8.5	9.84	0.77	10.5	8.1	7.0	10.7	8.7	8.0	9.9	10.1	9.11	0.45	24.5	0.4418
18-Feb-91	+1	10.7	9.2	5.4	12.2	7.6	7.3	8.5	7.8	8.56	0.70	10.1	8.1	6.1	10.5	6.0	9.0	5.8	4.9	7.54	0.72	-	-
25-Feb-91	+2	8.6	9.2	6.6	12.5	7.9	6.8	10.7	8.2	8.79	0.65	7.7	8.5	9.6	13.5	7.5	7.4	8.5	6.6	8.63	0.71	-	-
04-Mar-91	+3	9.6	7.7	7.8	9.9	8.1	8.2	11.4	7.9	8.81	0.44	11.9	8.8	8.6	7.0	9.3	6.3	10.4	7.9	8.75	0.59	-	-
11-Mar-91	+4	11.5	11.7	7.8	7.9	8.4	8.5	11.0	7.8	9.29	0.58	7.5	8.5	8.5	6.5	6.0	6.9	10.0	7.6	7.64	0.43	14.0	0.0650
18-Mar-91	+5	9.1	9.4	7.4	7.3	10.8	5.5	11.7	7.0	8.51	0.69	9.6	7.2	6.6	8.0	5.8	6.5	12.0	6.9	7.82	0.68	-	-
25-Mar-91	+6	10.0	11.2	5.0	9.4	7.7	7.9	9.5	9.5	8.75	0.63	11.2	9.6	7.4	11.0	5.0	6.5	9.3	7.1	8.36	0.74	-	-
01-Apr-91	+7	8.5	7.0	6.6	10.3	6.6	7.5	9.9	9.6	8.23	0.51	6.7	7.1	7.0	9.1	7.2	7.2	10.5	8.0	7.83	0.43	-	-
08-Apr-91	+8	13.0	7.0	8.5	9.3	12.4	5.3	12.4	6.8	9.30	0.98	7.7	7.1	6.3	12.6	7.7	8.4	7.5	8.1	8.14	0.63	26.0	0.5737
15-Apr-91	+9	7.1	10.0	7.3	11.0	5.6	8.1	7.8	7.2	8.00	0.57	10.4	10.2	5.8	7.5	5.7	7.4	14.3	8.4	8.69	0.95	-	-
22-Apr-91	+10	9.6	11.7	6.1	9.7	8.2	5.3	9.2	5.7	8.18	0.75	11.8	9.4	5.5	13.5	6.6	6.5	9.0	7.5	8.71	0.92	-	-
29-Apr-91	+11	7.8	8.4	9.2	12.9	6.6	4.5	10.9	5.9	8.24	0.90	11.5	11.4	8.9	7.4	5.5	7.7	7.2	7.3	8.34	0.70	-	-
06-May-91	+12	9.5	8.7	7.8	6.0	9.7	5.6	11.4	8.1	8.33	0.64	9.3	9.0	6.7	5.8	5.7	6.8	12.2	7.4	7.84	0.72	26.0	0.5737
13-May-91	+13	9.8	15.0	8.5	6.7	7.9	8.7	12.8	7.7	9.62	0.93	9.7	9.7	5.4	10.1	4.9	6.9	10.1	11.3	8.49	0.80	-	-
20-May-91	+14	8.3	5.0	6.9	6.0	8.9	5.3	6.5	6.8	6.69	0.44	10.6	11.5	7.9	8.5	7.2	6.7	6.4	4.0	7.84	0.79	-	-
27-May-91	+15	9.2	**	9.3	9.3	7.1	6.7	7.6	10.7	8.51	0.51	7.7	10.9	6.7	11.5	7.5	7.1	10.7	7.5	8.67	0.65	-	-
03-Jun-91	+16	9.1	**	7.5	9.2	6.0	**	7.9	6.6	7.71	0.48	9.0	8.6	8.4	9.3	7.1	5.4	8.4	7.4	7.95	0.42	22.0	0.8518
10-Jun-91	+17	7.7	**	6.8	8.2	6.3	**	6.5	8.4	7.28	0.34	8.3	10.2	6.4	8.5	6.7	6.4	7.4	5.2	7.38	0.52	-	-
17-Jun-91	+18	8.4	**	10.4	10.4	8.6	**	8.6	7.3	8.94	0.46	9.6	8.8	9.4	10.4	8.9	9.6	11.4	7.3	9.40	0.40	-	-
24-Jun-91	+19	7.7	**	**	8.0	**	**	7.9	7.2	7.70	0.15	9.0	8.0	8.1	8.4	6.2	6.0	7.6	5.6	7.36	0.42	-	-
01-Jul-91	+20	8.6	**	**	8.9	**	**	6.5	8.6	8.10	0.48	11.5	9.6	9.0	11.2	9.2	7.6	11.7	7.2	9.59	0.57	16.0	0.1091
08-Jul-91	+21	7.3	**	**	7.4	**	**	6.1	10.7	7.88	0.85	10.5	9.1	8.7	12.4	8.7	9.4	12.9	7.9	9.94	0.60	-	-
15-Jul-91	+22	9.1	**	**	6.9	**	**	5.5	7.1	7.17	0.64	10.6	9.5	9.9	11.4	8.7	9.0	9.6	7.2	9.48	0.42	-	-
22-Jul-91	+23	9.0	**	**	7.3	**	**	5.6	5.8	6.92	0.68	10.4	7.1	9.5	9.9	6.5	7.6	10.8	8.5	8.77	0.52	-	-
29-Jul-91	+24	7.7	**	**	7.8	**	**	6.9	10.8	8.30	0.75	11.3	10.6	10.3	11.8	8.1	8.4	10.8	8.6	10.00	0.47	5.5	0.0727
05-Aug-91	+25	10.6	**	**	9.7	**	**	6.9	7.4	8.67	0.77	9.5	11.5	9.2	10.5	7.4	8.5	10.9	8.8	9.54	0.45	-	-
12-Aug-91	+26	18.8	**	**	6.5	**	**	5.9	9.6	10.20	2.58	10.6	12.3	8.7	10.1	7.4	7.5	11.9	8.3	9.61	0.63	-	-
19-Aug-91	+27	**	**	**	6.9	**	**	**	6.7	6.80	0.09	10.6	10.2	10.9	9.6	6.4	7.8	10.8	7.6	9.25	0.57	-	-
26-Aug-91	+28	**	**	**	7.9	**	**	**	15.5	11.70	2.67	10.9	10.5	10.3	11.6	8.0	8.1	18.7	7.4	10.69	1.18	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead.

Appendix Table 10.4.12
Eosinophil Counts (x10⁹ cells/litre of blood) of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	0.19	0.16	0.02	0.01	0.06	0.01	0.1	0.1	0.07	0.02	0.06	0.05	0.04	0.08	0.05	0.01	0.06	0.0	0.05	0.01	-	-
07-Jan-91	-5	0.19	0.15	0.02	0.03	0.05	0.01	0.1	0.0	0.07	0.02	0.08	0.05	0.01	0.20	0.02	0.03	0.47	0.1	0.12	0.05	-	-
14-Jan-91	-4	0.17	0.18	0.11	0.02	0.03	0.01	0.15	0.01	0.09	0.02	0.02	0.06	0.02	0.18	0.02	0.01	0.17	0.05	0.07	0.02	30.5	0.8785
21-Jan-91	-3	0.07	0.18	0.07	0.03	0.03	0.01	0.07	0.04	0.06	0.02	0.02	0.04	0.01	0.07	0.01	0.01	0.35	0.02	0.07	0.04	-	-
28-Jan-91	-2	0.06	0.14	0.05	0.03	0.05	0.01	0.01	0.07	0.05	0.01	0.06	0.06	0.01	0.10	0.01	0.04	0.47	0.04	0.10	0.05	-	-
04-Feb-91	-1	0.10	0.07	0.03	0.01	0.03	0.01	0.05	0.16	0.06	0.02	0.03	0.11	0.01	0.10	0.02	0.05	0.40	0.02	0.09	0.04	-	-
11-Feb-91	0	0.13	0.18	0.07	0.01	0.05	0.02	0.04	0.10	0.07	0.02	0.04	0.06	0.03	0.12	0.02	0.08	0.08	0.02	0.06	0.01	27.5	0.6454
18-Feb-91	+1	0.27	0.10	0.07	0.06	0.07	0.04	0.13	0.07	0.10	0.02	0.04	0.21	0.02	0.08	0.02	0.07	0.09	0.01	0.07	0.02	21.0	0.2786
25-Feb-91	+2	0.86	0.58	0.31	0.16	0.55	0.14	0.23	0.21	0.38	0.08	0.04	0.04	0.02	0.10	0.06	0.21	0.28	0.01	0.09	0.03	6.5	0.0047
04-Mar-91	+3	0.96	1.28	0.83	0.23	1.57	0.30	0.69	0.23	0.76	0.17	0.03	0.18	0.03	0.05	0.01	0.25	0.32	0.01	0.11	0.04	13.0	0.0306
11-Mar-91	+4	0.90	1.28	0.56	0.26	0.92	0.26	0.48	0.29	0.62	0.12	0.05	0.28	0.03	0.08	0.01	0.08	0.21	0.02	0.09	0.03	2.0	0.0006
18-Mar-91	+5	0.67	1.36	0.33	0.19	0.98	0.22	0.40	0.31	0.56	0.14	0.03	0.08	0.03	0.08	0.01	0.03	0.19	0.01	0.06	0.02	-	-
25-Mar-91	+6	0.98	0.66	0.51	0.21	0.36	0.17	0.35	0.30	0.44	0.09	0.06	0.38	0.05	0.11	0.05	0.07	0.11	0.02	0.11	0.04	-	-
01-Apr-91	+7	0.30	0.69	0.15	0.08	0.21	0.02	0.18	0.42	0.26	0.07	0.03	0.17	0.03	0.04	0.02	0.02	0.04	0.01	0.05	0.02	-	-
08-Apr-91	+8	0.53	0.88	0.22	0.06	0.18	0.05	0.08	0.17	0.27	0.10	0.04	0.08	0.00	0.05	0.03	0.02	0.03	0.01	0.03	0.01	3.0	0.0011
15-Apr-91	+9	0.44	1.40	0.70	0.23	0.25	0.12	0.13	0.27	0.44	0.14	0.09	0.19	0.05	0.04	0.01	0.06	0.07	0.02	0.07	0.02	-	-
22-Apr-91	+10	0.75	1.44	0.88	0.27	0.55	0.22	0.22	0.24	0.57	0.14	0.12	0.20	0.04	0.17	0.05	0.15	0.17	0.03	0.12	0.02	-	-
29-Apr-91	+11	1.18	0.77	0.87	0.12	0.22	0.14	0.23	0.43	0.50	0.13	0.14	0.31	0.12	0.16	0.06	0.13	0.10	0.07	0.14	0.03	-	-
06-May-91	+12	1.03	1.34	1.10	0.08	0.05	0.20	0.08	0.28	0.52	0.18	0.06	0.28	0.03	0.13	0.03	0.05	0.11	0.07	0.09	0.03	13.0	0.0499
13-May-91	+13	1.55	2.34	0.91	0.21	0.08	0.18	0.16	0.58	0.75	0.27	0.06	0.23	0.23	0.16	0.05	0.18	0.03	0.06	0.12	0.03	12.0	0.0379
20-May-91	+14	1.22	0.05	1.21	0.33	0.08	0.06	0.30	0.33	0.45	0.16	0.04	0.26	0.13	0.15	0.04	0.15	0.07	0.05	0.11	0.02	14.5	0.0650
27-May-91	+15	1.39	**	0.84	0.21	0.14	0.08	0.10	0.25	0.43	0.17	0.10	0.27	0.03	0.12	0.03	0.16	0.09	0.05	0.11	0.03	12.5	0.0721
03-Jun-91	+16	1.41	**	0.09	0.29	0.11	**	0.32	0.05	0.38	0.19	0.12	0.18	0.12	0.17	0.07	0.08	0.19	0.04	0.12	0.02	17.0	0.4136
10-Jun-91	+17	1.32	**	0.24	0.45	0.11	**	0.35	0.33	0.47	0.16	0.14	0.26	0.05	0.09	0.08	0.18	0.15	0.03	0.12	0.03	-	-
17-Jun-91	+18	0.98	**	0.02	0.32	0.02	**	0.36	0.34	0.34	0.13	0.05	0.12	0.11	0.08	0.06	0.15	0.13	0.06	0.09	0.01	-	-
24-Jun-91	+19	0.61	**	**	0.17	**	**	1.33	0.02	0.53	0.25	0.04	0.05	0.11	0.13	0.02	0.12	0.14	0.18	0.10	0.02	-	-
01-Jul-91	+20	0.18	**	**	0.12	**	**	0.09	0.26	0.16	0.03	0.03	0.11	0.16	0.12	0.04	0.08	0.10	0.11	0.09	0.01	6.5	0.1091
08-Jul-91	+21	0.73	**	**	0.09	**	**	0.04	0.26	0.28	0.14	0.04	0.14	0.08	0.17	0.05	0.08	0.17	0.05	0.10	0.02	-	-
15-Jul-91	+22	0.19	**	**	0.08	**	**	0.03	0.15	0.11	0.03	0.08	0.06	0.06	0.15	0.01	0.09	0.14	0.14	0.09	0.02	-	-
22-Jul-91	+23	0.39	**	**	0.06	**	**	0.05	0.17	0.17	0.07	0.09	0.15	0.05	0.14	0.01	0.10	0.16	0.08	0.10	0.02	-	-
29-Jul-91	+24	0.34	**	**	0.06	**	**	0.01	0.38	0.20	0.08	0.13	0.13	0.03	0.16	0.03	0.13	0.17	0.12	0.11	0.02	14.0	0.8081
05-Aug-91	+25	0.14	**	**	0.05	**	**	0.01	0.16	0.09	0.03	0.13	0.12	0.06	0.10	0.03	0.16	0.17	0.08	0.11	0.02	-	-
12-Aug-91	+26	0.04	**	**	0.09	**	**	0.02	0.06	0.05	0.01	0.21	0.06	0.08	0.18	0.02	0.03	0.14	0.04	0.10	0.02	-	-
19-Aug-91	+27	**	**	**	0.13	**	**	**	0.15	0.14	0.01	0.19	0.09	0.13	0.13	0.06	0.12	0.13	0.11	0.12	0.01	-	-
26-Aug-91	+28	**	**	**	0.15	**	**	**	0.18	0.16	0.01	0.13	0.16	0.09	0.16	0.16	0.18	0.14	0.15	0.15	0.01	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead;

the weeks when the median values of infected and uninfected groups were significantly different are printed in bold faces

Appendix Table 10.4.13
Serum Total Protein (g/l) of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	64.0	59.7	65.4	61.4	64.2	60.5	56.4	60.2	61.48	0.97	67.0	62.3	63.0	61.5	60.5	64.6	69.7	54.7	62.91	1.49	-	-
07-Jan-91	-5	65.3	59.0	63.7	59.4	68.7	58.4	59.9	58.8	61.65	1.26	67.0	65.7	68.0	64.3	66.6	66.7	59.4	56.8	64.31	1.34	-	-
14-Jan-91	-4	64.0	56.7	62.3	60.2	64.5	56.9	54.2	66.6	60.68	1.46	60.7	59.3	62.2	58.8	59.9	60.8	61.3	58.7	60.21	0.41	28.0	0.7209
21-Jan-91	-3	69.0	58.4	68.0	65.3	72.2	60.0	59.4	62.0	64.29	1.68	67.6	62.3	65.0	69.2	61.7	67.1	66.0	59.4	64.78	1.11	-	-
28-Jan-91	-2	69.1	56.7	63.9	59.8	66.6	58.4	58.2	66.6	62.41	1.56	59.1	61.9	68.6	59.9	59.2	69.2	60.9	53.9	61.59	1.68	-	-
04-Feb-91	-1	68.4	60.8	64.5	59.0	59.5	55.7	58.9	61.1	60.99	1.28	67.4	61.9	66.8	60.6	55.1	61.6	63.7	70.2	63.40	1.56	-	-
11-Feb-91	0	66.8	59.6	62.3	60.4	58.7	50.6	59.6	69.3	60.90	1.87	66.8	62.4	61.1	53.3	67.9	64.7	50.6	59.4	60.76	2.04	30.0	0.8785
18-Feb-91	+1	69.6	58.6	64.7	61.0	67.4	57.6	60.3	66.1	63.16	1.44	66.8	56.9	64.9	64.2	61.0	63.2	67.4	60.8	63.15	1.15	-	-
25-Feb-91	+2	68.9	67.4	65.1	61.1	67.4	56.3	61.1	68.7	64.51	1.50	71.9	66.4	73.5	61.1	57.6	62.9	70.4	60.8	65.58	1.94	-	-
04-Mar-91	+3	68.0	64.7	62.9	61.1	67.4	56.1	60.3	70.4	63.87	1.56	71.9	68.7	69.7	67.4	57.9	61.1	69.7	61.1	65.94	1.70	-	-
11-Mar-91	+4	73.0	63.8	68.5	68.0	65.5	57.3	59.5	74.5	66.25	2.00	71.5	61.5	68.0	71.8	55.8	61.8	70.3	63.5	65.50	1.91	28.5	0.7209
18-Mar-91	+5	73.0	65.7	65.9	70.0	67.4	53.5	67.1	73.2	66.98	2.05	70.5	66.6	67.3	61.0	56.7	65.7	55.8	60.3	62.97	1.77	-	-
25-Mar-91	+6	67.4	65.7	66.2	63.9	60.6	50.1	62.6	69.7	63.26	2.00	63.2	61.5	66.6	63.1	61.1	65.9	62.9	62.5	63.34	0.65	-	-
01-Apr-91	+7	72.5	73.9	69.5	64.2	65.9	59.8	60.3	68.3	66.80	1.73	70.5	66.4	60.3	62.5	63.5	64.9	68.6	56.2	64.10	1.52	-	-
08-Apr-91	+8	75.6	74.7	69.4	69.4	72.9	56.5	70.9	64.7	69.25	2.05	73.5	68.2	65.3	66.5	65.3	69.3	65.9	67.9	67.74	0.91	20.0	0.2345
15-Apr-91	+9	77.2	66.9	75.2	71.2	74.1	61.9	75.0	65.3	70.84	1.83	71.8	64.1	68.4	67.9	63.1	63.2	67.5	66.6	66.58	0.99	-	-
22-Apr-91	+10	74.7	67.9	72.7	70.4	74.5	68.7	71.1	64.7	70.59	1.14	74.0	68.9	68.0	67.5	64.6	66.5	69.7	56.7	67.00	1.65	-	-
29-Apr-91	+11	71.4	65.0	74.6	69.6	69.3	67.7	69.1	64.1	68.86	1.11	69.6	68.6	66.8	65.4	65.4	66.1	69.7	69.9	67.68	0.66	28.0	0.7209
06-May-91	**+12**	73.7	67.8	70.6	66.4	66.2	64.7	64.9	75.6	68.73	1.36	64.9	63.8	70.4	66.0	61.8	60.1	65.1	63.4	64.43	1.02	10.5	0.0207
13-May-91	**+13**	74.6	68.1	64.6	66.9	68.1	65.8	69.2	78.9	69.53	1.60	69.4	61.2	61.2	64.4	61.4	64.0	65.8	61.9	63.65	0.95	7.5	0.0070
20-May-91	**+14**	69.5	65.2	68.4	69.1	69.1	58.3	69.8	75.6	68.14	1.62	63.8	66.1	63.8	62.2	62.4	67.1	62.7	55.7	62.97	1.13	9.0	0.0148
27-May-91	+15	66.0	**	64.1	65.8	62.2	50.2	73.9	79.8	65.99	3.26	72.0	67.6	68.3	63.8	59.9	63.2	60.3	60.6	64.46	1.46	22.0	0.5358
03-Jun-91	+16	64.6	**	57.5	62.2	63.5	**	72.2	75.6	65.94	2.50	68.2	63.7	63.7	62.5	61.3	66.4	60.2	59.1	63.13	1.02	19.0	0.5728
10-Jun-91	+17	55.2	**	48.5	59.5	47.4	**	64.3	73.2	58.01	3.66	68.6	62.1	60.4	63.8	62.4	61.1	62.7	59.3	62.54	0.93	-	-
17-Jun-91	+18	60.0	**	46.3	54.5	43.7	**	61.3	70.3	55.99	3.71	70.3	59.8	67.5	62.2	64.0	63.2	66.3	60.6	64.23	1.18	-	-
24-Jun-91	+19	55.2	**	**	53.6	**	**	57.9	62.5	57.30	1.68	60.7	63.7	66.2	64.4	59.9	61.9	65.8	64.4	63.38	0.76	-	-
01-Jul-91	+20	52.0	**	**	50.2	**	**	54.0	66.4	55.67	3.18	62.0	64.0	63.8	64.2	60.4	65.3	69.7	70.9	65.04	1.18	6.0	0.1091
08-Jul-91	+21	47.9	**	**	50.4	**	**	50.7	59.5	52.13	2.20	63.7	63.9	61.5	61.5	54.4	61.7	62.1	63.9	61.59	1.02	-	-
15-Jul-91	+22	46.9	**	**	51.7	**	**	49.4	63.7	52.91	3.22	66.5	56.2	60.4	59.8	60.7	65.7	66.0	63.4	62.33	1.21	-	-
22-Jul-91	+23	39.9	**	**	41.0	**	**	44.3	60.9	46.53	4.22	61.8	60.2	67.2	61.3	56.4	66.7	61.3	59.2	61.78	1.20	-	-
29-Jul-91	+24	39.9	**	**	32.3	**	**	33.6	60.9	41.69	5.73	58.5	58.4	60.4	62.3	57.4	59.0	58.5	55.9	58.82	0.63	7.0	0.1535
05-Aug-91	+25	36.6	**	**	48.5	**	**	39.3	53.9	44.57	3.46	62.6	63.2	55.4	67.0	59.2	67.7	64.6	57.0	62.08	1.49	-	-
12-Aug-91	+26	38.6	**	**	43.0	**	**	34.2	58.1	43.45	4.50	64.0	61.4	61.4	60.9	60.1	62.2	62.2	60.5	61.58	0.41	-	-
19-Aug-91	+27	**	**	**	39.6	**	**	**	54.6	47.10	5.33	67.1	69.3	57.9	61.0	59.7	61.6	68.2	60.8	63.19	1.43	-	-
26-Aug-91	+28	**	**	**	41.9	**	**	**	54.2	48.04	4.34	64.3	60.6	67.7	62.7	58.3	54.2	62.9	62.5	61.64	1.33	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead;

the weeks when the median values of infected and uninfected groups were significantly different are printed in bold faces.

Appendix Table 10.4.14
Serum Albumun Levels (g/l) of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	31.6	25.2	27.5	31.4	29.8	28.8	31.5	30.2	29.50	0.75	29.2	30.8	27.8	28.6	30.5	29.9	31.6	28.8	29.65	0.42	-	-
07-Jan-91	-5	30.0	26.2	27.8	25.6	27.9	29.0	29.8	31.4	28.46	0.65	29.4	31.9	21.9	31.0	28.5	31.0	31.9	26.7	29.04	1.12	-	-
14-Jan-91	-4	32.5	28.0	30.4	33.7	30.8	25.2	32.3	29.2	30.26	0.91	28.0	38.0	33.1	33.3	32.4	25.5	31.1	33.6	31.88	1.25	21.5	0.2786
21-Jan-91	-3	34.6	29.5	27.7	31.1	28.7	24.8	31.5	32.4	30.04	1.01	31.9	29.5	29.5	32.8	24.4	27.1	27.3	25.0	28.43	1.00	-	-
28-Jan-91	-2	31.1	28.8	27.1	24.8	32.3	27.5	28.6	27.7	28.48	0.77	24.6	27.2	31.1	32.2	22.9	24.4	27.6	26.7	27.08	1.07	-	-
04-Feb-91	-1	24.4	32.8	27.4	32.7	26.7	26.2	32.7	30.8	29.19	1.12	27.9	31.7	39.6	35.8	30.2	27.2	30.5	29.7	31.57	1.37	-	-
11-Feb-91	0	25.4	26.5	31.5	24.2	29.8	27.8	27.2	31.4	27.96	0.89	31.0	32.6	25.5	31.4	28.9	26.3	27.4	28.0	28.88	0.84	25.5	0.5054
18-Feb-91	+1	25.5	26.1	27.0	29.7	25.5	29.2	32.1	31.9	28.36	0.91	28.0	34.0	29.7	28.2	25.9	24.1	29.5	25.2	28.07	1.03	-	-
25-Feb-91	+2	22.1	29.0	26.2	29.5	29.5	24.5	32.7	28.4	27.72	1.10	26.3	37.9	32.7	29.5	30.7	28.5	30.0	35.2	31.36	1.25	-	-
04-Mar-91	+3	28.5	29.0	28.5	29.9	29.8	26.8	32.7	26.3	28.95	0.66	29.0	32.7	29.9	29.0	26.8	28.4	32.7	32.7	30.17	0.76	-	-
11-Mar-91	+4	23.3	30.7	26.6	30.1	28.0	26.0	30.8	24.5	27.51	0.95	30.8	31.4	28.1	29.1	25.0	25.0	37.3	30.4	29.63	1.31	20.5	0.2345
18-Mar-91	+5	24.4	26.7	30.5	33.9	30.1	29.1	30.2	27.2	29.01	0.95	29.8	24.1	32.0	33.0	30.1	38.0	31.3	31.8	31.26	1.27	-	-
25-Mar-91	+6	21.8	29.5	24.0	30.8	24.9	23.4	29.9	22.2	25.80	1.22	28.5	31.6	28.7	30.2	30.4	22.9	26.8	29.9	28.63	0.90	-	-
01-Apr-91	+7	24.3	33.9	25.9	26.4	28.9	26.8	32.7	23.0	27.74	1.28	29.5	32.4	28.7	24.7	37.4	29.4	27.5	29.9	29.94	1.23	-	-
08-Apr-91	+8	31.5	31.3	36.4	26.4	29.3	27.6	36.9	23.1	30.32	1.57	31.9	35.0	34.3	33.8	32.1	29.3	30.1	30.1	32.07	0.70	22.5	0.3282
15-Apr-91	+9	30.2	28.4	32.0	24.4	28.6	33.8	34.8	29.6	30.23	1.10	32.2	39.0	35.6	28.6	31.0	29.8	29.4	29.8	31.93	1.19	-	-
22-Apr-91	+10	27.0	25.9	23.6	31.8	24.9	27.7	34.1	30.9	28.22	1.22	32.6	34.2	29.6	32.6	34.5	27.4	33.9	32.0	32.10	0.81	-	-
29-Apr-91	+11	33.3	32.4	32.0	32.1	31.2	26.1	31.1	31.9	31.26	0.73	32.8	37.1	33.1	30.7	32.1	29.4	30.4	30.8	32.06	0.80	-	-
06-May-91	+12	29.9	31.0	30.0	31.9	31.8	24.3	30.2	31.3	30.06	0.81	28.1	30.3	30.4	29.0	33.8	34.5	29.6	24.3	30.00	1.06	27.5	0.6454
13-May-91	**+13**	31.1	29.9	26.6	32.5	26.1	30.7	32.2	31.7	30.11	0.82	34.8	32.1	33.3	32.6	33.5	32.7	30.4	34.9	33.04	0.48	7.0	0.0070
20-May-91	**+14**	24.6	20.7	19.6	28.4	19.2	19.9	26.0	27.4	23.22	1.26	29.8	33.8	26.8	38.5	37.5	26.8	30.8	34.9	32.36	1.49	4.0	0.0019
27-May-91	**+15**	17.8	**	13.3	18.8	13.0	18.2	19.6	17.3	16.85	0.92	26.8	34.4	26.8	37.6	28.0	25.7	27.2	33.4	29.99	1.48	0.0	0.0003
03-Jun-91	**+16**	21.8	**	15.8	23.0	19.5	**	23.2	21.6	20.81	1.04	30.1	33.9	33.5	29.4	31.9	29.2	30.9	34.6	31.69	0.70	0.0	0.0007
10-Jun-91	**+17**	25.2	**	13.7	23.6	15.3	**	20.0	23.1	20.16	1.75	36.9	27.3	35.0	38.0	31.6	39.0	24.5	35.5	33.47	1.72	-	-
17-Jun-91	**+18**	21.1	**	14.1	21.0	17.3	**	21.8	17.7	18.83	1.11	34.4	38.0	36.9	37.6	33.7	31.0	30.8	32.4	34.35	0.95	-	-
24-Jun-91	**+19**	25.3	**	**	20.0	**	**	19.2	17.1	20.41	1.52	30.8	27.1	31.4	34.2	28.1	25.9	30.1	24.4	29.00	1.07	-	-
01-Jul-91	**+20**	23.6	**	**	27.7	**	**	15.7	14.6	20.40	2.73	40.8	25.6	28.9	29.1	28.0	28.2	30.3	37.7	31.08	1.75	1.0	0.0081
08-Jul-91	**+21**	16.4	**	**	23.1	**	**	15.6	21.5	19.12	1.61	22.5	31.1	31.1	25.9	36.0	23.2	37.0	27.4	29.25	1.81	-	-
15-Jul-91	**+22**	18.0	**	**	23.1	**	**	23.7	15.2	20.00	1.77	27.2	34.1	29.0	26.7	28.3	26.9	30.2	30.0	29.04	0.81	-	-
22-Jul-91	**+23**	12.7	**	**	17.0	**	**	16.9	19.0	16.38	1.14	25.0	28.7	27.0	29.7	27.8	24.6	27.3	28.4	27.32	0.58	-	-
29-Jul-91	**+24**	15.1	**	**	13.0	**	**	14.9	19.3	15.59	1.14	24.5	29.8	28.8	27.0	25.5	25.5	25.5	26.3	26.59	0.60	0.0	0.0040
05-Aug-91	+25	13.7	**	**	19.2	**	**	18.8	21.0	18.15	1.36	35.4	31.3	25.0	33.0	30.3	27.0	28.0	33.2	30.39	1.17	-	-
12-Aug-91	+26	13.9	**	**	14.5	**	**	13.1	19.5	15.23	1.24	27.4	27.8	28.6	27.5	37.5	25.4	29.5	29.5	29.13	1.20	-	-
19-Aug-91	+27	**	**	**	17.5	**	**	**	23.2	20.38	2.92	27.5	25.7	27.9	30.5	28.0	26.4	27.5	28.5	27.74	0.47	-	-
26-Aug-91	+28	**	**	**	18.5	**	**	**	21.7	20.07	1.14	30.3	24.3	37.0	30.9	29.4	26.4	32.5	27.8	29.84	1.29	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead.

the weeks when the median values of infected and uninfected groups were significantly different are printed in bold faces.

Appendix Table 10.4.15
Serum Globulins (g/l) of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	32.4	34.5	37.9	30.0	34.4	31.7	24.9	30.0	31.98	1.28	37.8	31.5	35.2	32.9	30.0	34.7	38.1	25.9	33.26	1.36	-	-
07-Jan-91	-5	35.3	32.8	35.9	33.8	40.8	29.4	30.1	27.4	33.19	1.41	37.6	33.8	46.1	33.3	38.1	35.7	27.5	30.1	35.28	1.87	-	-
14-Jan-91	-4	31.5	28.7	31.9	26.5	33.7	31.7	21.9	37.4	30.41	1.56	32.7	21.3	29.1	25.5	27.5	35.3	30.2	25.1	28.34	1.48	23.0	0.3823
21-Jan-91	-3	34.4	28.9	40.3	34.2	43.5	35.2	27.9	29.6	34.26	1.83	35.7	32.8	35.5	36.4	37.3	40.0	38.6	34.4	36.35	0.76	-	-
28-Jan-91	-2	38.0	27.9	36.8	35.0	34.3	30.9	29.6	38.9	33.92	1.34	34.6	34.7	37.5	27.7	36.3	44.7	33.3	27.3	34.51	1.84	-	-
04-Feb-91	-1	44.0	28.1	37.1	26.4	32.9	29.4	26.3	30.4	31.80	2.02	39.5	30.2	27.2	24.8	24.9	34.4	33.2	40.5	31.84	2.03	-	-
11-Feb-91	0	41.4	33.1	30.8	36.2	28.9	22.8	32.5	38.0	32.94	1.90	35.8	29.8	35.6	22.0	39.1	38.4	23.2	31.3	31.88	2.17	30.0	0.8785
18-Feb-91	+1	44.1	32.5	37.7	31.3	41.9	28.5	28.2	34.3	34.80	1.96	38.9	22.9	35.2	36.0	35.2	39.1	37.9	35.6	35.09	1.71	-	-
25-Feb-91	+2	46.9	38.4	38.9	31.6	38.0	31.8	28.4	40.3	36.79	1.95	45.6	28.5	40.7	31.6	26.9	34.4	40.4	25.6	34.22	2.43	-	-
04-Mar-91	+3	39.5	35.7	34.4	31.3	37.6	29.3	27.6	44.1	34.92	1.83	42.9	35.9	39.8	38.4	31.0	32.8	36.9	28.4	35.78	1.59	-	-
11-Mar-91	+4	49.7	33.0	41.9	37.9	37.5	31.2	28.8	50.0	38.75	2.65	40.8	30.1	39.9	42.7	30.8	36.8	33.0	33.1	35.87	1.59	26.5	0.5737
18-Mar-91	+5	48.6	39.0	35.4	36.2	37.3	24.4	36.9	46.0	37.97	2.41	40.7	42.5	35.3	27.9	26.6	37.8	34.5	28.5	34.22	2.00	-	-
25-Mar-91	+6	45.6	36.2	42.2	33.1	35.7	26.6	32.7	47.6	37.46	2.35	34.7	29.9	37.9	32.9	30.6	43.0	36.1	32.5	34.70	1.42	-	-
01-Apr-91	+7	48.2	40.0	43.6	37.8	37.0	33.0	27.6	45.4	39.07	2.23	41.0	34.0	31.6	37.8	26.1	35.5	41.1	26.3	34.16	1.95	-	-
08-Apr-91	+8	44.1	43.4	33.0	43.0	43.7	28.8	33.9	41.6	38.93	2.00	41.6	33.2	31.0	32.7	33.2	40.0	35.8	37.8	35.67	1.26	18.5	0.1605
15-Apr-91	+9	47.0	38.5	43.2	46.8	45.5	28.1	40.2	35.7	40.61	2.15	39.6	25.1	32.8	39.3	32.1	33.4	38.1	36.8	34.65	1.61	-	-
22-Apr-91	+10	47.7	42.0	49.1	38.6	49.7	41.0	37.0	33.8	42.37	1.96	41.4	34.8	38.4	34.9	30.1	39.1	35.8	24.7	34.89	1.77	-	-
29-Apr-91	+11	38.1	32.6	42.6	37.5	38.1	41.6	38.0	32.2	37.60	1.22	36.8	31.5	33.7	34.7	33.3	36.7	39.3	39.1	35.63	0.93	-	-
06-May-91	+12	43.8	36.8	40.6	34.5	34.3	40.4	34.7	44.3	38.67	1.37	36.9	33.5	40.0	37.0	28.0	25.6	35.5	39.1	34.43	1.71	19.0	0.1949
13-May-91	+13	43.5	38.2	38.1	34.4	42.0	35.1	37.0	47.2	39.43	1.47	34.6	29.1	27.9	31.8	27.9	31.3	35.4	27.0	30.62	1.05	3.0	0.0011
20-May-91	+14	45.0	44.6	48.8	40.7	50.0	38.4	43.8	48.2	44.92	1.33	34.0	32.3	37.0	23.7	25.0	40.3	31.8	20.8	30.62	2.25	1.0	0.0003
27-May-91	+15	48.2	**	50.8	47.0	49.2	31.9	54.4	62.5	49.14	3.23	45.2	33.2	41.6	26.2	31.9	37.5	33.0	27.2	34.47	2.19	5.5	0.0059
03-Jun-91	+16	42.9	**	41.7	39.2	44.0	**	49.0	54.0	45.13	2.02	38.1	29.8	30.3	33.1	29.4	37.2	29.2	24.5	31.44	1.49	0.0	0.0007
10-Jun-91	+17	29.9	**	34.8	35.9	32.1	**	44.3	50.1	37.84	2.89	31.7	34.8	25.4	25.8	30.8	22.1	38.2	23.8	29.07	1.88	8.5	0.0426
17-Jun-91	+18	38.9	**	32.1	33.6	26.4	**	39.5	52.6	37.17	3.33	35.9	21.7	30.6	24.6	30.3	32.2	35.5	28.2	29.87	1.63	11.0	0.1079
24-Jun-91	+19	29.9	**	**	33.6	**	**	38.7	45.4	36.89	2.91	29.9	36.6	34.8	30.2	31.8	36.0	35.7	40.0	34.38	1.16	13.5	0.6828
01-Jul-91	+20	28.4	**	**	22.5	**	**	38.3	51.9	35.27	5.56	21.2	38.4	34.9	35.1	32.5	37.1	39.4	33.1	33.97	1.88	16.0	1.0667
08-Jul-91	+21	31.6	**	**	27.3	**	**	35.1	38.0	33.01	2.00	41.2	32.9	30.5	35.6	18.4	38.5	25.2	36.5	32.34	2.49	-	-
15-Jul-91	+22	28.9	**	**	28.5	**	**	25.7	48.5	32.91	4.54	39.2	22.1	31.5	33.1	32.4	38.9	35.8	33.4	33.28	1.78	-	-
22-Jul-91	+23	27.2	**	**	24.1	**	**	27.4	41.9	30.15	3.46	36.8	31.5	40.2	31.7	28.6	42.1	34.0	30.8	34.46	1.59	-	-
29-Jul-91	+24	24.8	**	**	19.3	**	**	18.7	41.6	26.10	4.64	34.1	28.7	31.6	35.3	31.9	33.6	33.0	29.6	32.22	0.74	8.0	0.2141
05-Aug-91	+25	23.0	**	**	29.3	**	**	20.6	32.9	26.43	2.45	27.2	32.0	30.4	34.0	28.9	40.8	36.6	23.8	31.69	1.79	-	-
12-Aug-91	+26	24.7	**	**	28.5	**	**	21.1	38.6	28.22	3.28	36.7	33.5	32.8	33.5	22.6	36.9	32.8	31.0	32.46	1.48	-	-
19-Aug-91	+27	**	**	**	22.0	**	**	**	31.4	26.72	3.31	39.7	43.6	30.0	30.5	31.6	35.2	40.7	32.3	35.45	1.73	-	-
26-Aug-91	+28	**	**	**	18.5	**	**	**	32.5	25.47	4.96	34.0	36.3	30.6	31.8	28.9	27.8	30.4	34.7	31.80	0.98	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead;

the weeks when the median values of the infected and uninfectcd were significantly different are printed in bold faces.

Appendix Table 10.4.16
Serum Albumun to Serum Globulin Ratios of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
31-Dec-90	-6	0.98	0.73	0.73	1.05	0.87	0.91	1.27	1.01	0.94	0.06	0.77	0.98	0.79	0.87	1.02	0.86	0.83	1.11	0.90	0.04	-	-
07-Jan-91	-5	0.85	0.80	0.77	0.76	0.68	0.99	0.99	1.15	0.87	0.05	0.78	0.94	0.48	0.93	0.75	0.87	1.16	0.89	0.85	0.06	-	-
14-Jan-91	-4	1.03	0.98	0.95	1.27	0.91	0.79	1.47	0.78	1.02	0.08	0.86	1.78	1.14	1.31	1.18	0.72	1.03	1.34	1.17	0.11	22.5	0.3282
21-Jan-91	-3	1.01	1.02	0.69	0.91	0.66	0.70	1.13	1.09	0.90	0.06	0.89	0.90	0.83	0.90	0.65	0.68	0.71	0.73	0.79	0.04	-	-
28-Jan-91	-2	0.82	1.03	0.74	0.71	0.94	0.89	0.97	0.71	0.85	0.04	0.71	0.78	0.83	1.16	0.63	0.55	0.83	0.98	0.81	0.06	-	-
04-Feb-91	-1	0.55	1.17	0.74	1.24	0.81	0.89	1.24	1.01	0.96	0.08	0.71	1.05	1.45	1.44	1.21	0.79	0.92	0.73	1.04	0.10	-	-
11-Feb-91	0	0.61	0.80	1.02	0.67	1.03	1.22	0.84	0.83	0.88	0.07	0.87	1.09	0.72	1.43	0.74	0.68	1.18	0.89	0.95	0.09	26.0	0.5737
18-Feb-91	+1	0.58	0.80	0.72	0.95	0.61	1.02	1.14	0.93	0.84	0.07	0.72	1.48	0.84	0.79	0.74	0.62	0.78	0.71	0.83	0.09	-	-
25-Feb-91	+2	0.47	0.76	0.67	0.93	0.78	0.77	1.15	0.70	0.78	0.07	0.58	1.33	0.80	0.94	1.14	0.83	0.74	1.38	0.97	0.10	-	-
04-Mar-91	+3	0.72	0.81	0.83	0.95	0.79	0.92	1.19	0.60	0.85	0.06	0.68	0.91	0.75	0.76	0.87	0.86	0.89	1.15	0.86	0.05	-	-
11-Mar-91	+4	0.47	0.93	0.64	0.79	0.75	0.83	1.07	0.49	0.75	0.07	0.75	1.04	0.71	0.68	0.81	0.68	1.13	0.92	0.84	0.06	25.5	0.5054
18-Mar-91	+5	0.50	0.69	0.86	0.94	0.81	1.19	0.82	0.59	0.80	0.07	0.73	0.57	0.90	1.18	1.13	1.01	0.91	1.12	0.94	0.07	-	-
25-Mar-91	+6	0.48	0.81	0.57	0.93	0.70	0.88	0.91	0.47	0.72	0.06	0.82	1.06	0.76	0.92	0.99	0.53	0.74	0.92	0.84	0.05	-	-
01-Apr-91	+7	0.50	0.85	0.59	0.70	0.78	0.81	1.18	0.51	0.74	0.07	0.72	0.95	0.91	0.65	1.44	0.83	0.67	1.14	0.91	0.09	-	-
08-Apr-91	+8	0.71	0.72	1.10	0.61	0.67	0.96	1.09	0.56	0.80	0.07	0.77	1.05	1.10	1.03	0.96	0.73	0.84	0.80	0.91	0.05	19.0	0.1949
15-Apr-91	+9	0.64	0.74	0.74	0.52	0.63	1.20	0.87	0.83	0.77	0.07	0.81	1.55	1.09	0.73	0.97	0.89	0.77	0.81	0.95	0.09	-	-
22-Apr-91	+10	0.57	0.62	0.48	0.82	0.50	0.68	0.92	0.91	0.69	0.06	0.79	0.98	0.77	0.93	1.14	0.70	0.95	1.30	0.95	0.07	-	-
29-Apr-91	+11	0.87	1.00	0.75	0.86	0.82	0.63	0.82	0.99	0.84	0.04	0.89	1.18	0.98	0.89	0.96	0.80	0.77	0.79	0.91	0.04	-	-
06-May-91	+12	0.68	0.84	0.74	0.93	0.93	0.60	0.87	0.71	0.79	0.04	0.76	0.91	0.76	0.78	1.21	1.35	0.83	0.62	0.90	0.08	25.0	0.5054
13-May-91	+13	0.71	0.78	0.70	0.95	0.62	0.87	0.87	0.67	0.77	0.04	1.01	1.10	1.19	1.02	1.20	1.05	0.86	1.29	1.09	0.05	3.0	0.0011
20-May-91	+14	0.55	0.46	0.40	0.70	0.38	0.52	0.59	0.57	0.52	0.03	0.88	1.05	0.72	1.62	1.50	0.67	0.97	1.67	1.13	0.13	1.0	0.0003
27-May-91	+15	0.37	**	0.26	0.40	0.26	0.57	0.36	0.28	0.36	0.04	0.59	1.04	0.64	1.44	0.88	0.68	0.82	1.23	0.92	0.10	0.0	0.0003
03-Jun-91	+16	0.51	**	0.38	0.59	0.44	**	0.47	0.40	0.46	0.03	0.79	1.14	1.11	0.89	1.09	0.79	1.06	1.42	1.03	0.07	0.0	0.0007
10-Jun-91	+17	0.84	**	0.39	0.66	0.48	**	0.45	0.46	0.55	0.06	1.16	0.79	1.38	1.47	1.03	1.76	0.64	1.49	1.22	0.13	3.0	0.0047
17-Jun-91	+18	0.54	**	0.44	0.62	0.66	**	0.55	0.34	0.53	0.04	0.96	1.75	1.21	1.52	1.12	0.96	0.87	1.15	1.19	0.10	0.0	0.0007
24-Jun-91	+19	0.85	**	**	0.60	**	**	0.50	0.38	0.58	0.09	1.03	0.74	0.90	1.14	0.88	0.72	0.84	0.61	0.86	0.06	4.0	0.0485
01-Jul-91	+20	0.83	**	**	1.23	**	**	0.41	0.28	0.69	0.19	1.93	0.67	0.83	0.83	0.86	0.76	0.77	1.14	0.97	0.14	11.0	0.4606
08-Jul-91	+21	0.52	**	**	0.84	**	**	0.44	0.56	0.59	0.08	0.54	0.95	1.02	0.73	1.96	0.60	1.47	0.75	1.00	0.16	5.0	0.0727
15-Jul-91	+22	0.62	**	**	0.81	**	**	0.92	0.31	0.67	0.12	0.69	1.55	0.92	0.81	0.87	0.69	0.84	0.90	0.91	0.09	9.0	0.2828
22-Jul-91	+23	0.47	**	**	0.71	**	**	0.61	0.45	0.56	0.05	0.68	0.91	0.67	0.94	0.97	0.58	0.80	0.92	0.81	0.05	4.0	0.0485
29-Jul-91	+24	0.61	**	**	0.68	**	**	0.80	0.46	0.64	0.06	0.72	1.04	0.91	0.76	0.80	0.76	0.77	0.89	0.83	0.04	4.5	0.0485
05-Aug-91	+25	0.60	**	**	0.66	**	**	0.91	0.64	0.70	0.06	1.30	0.98	0.82	0.97	1.05	0.66	0.76	1.40	0.99	0.08	-	-
12-Aug-91	+26	0.55	**	**	0.51	**	**	0.62	0.50	0.55	0.02	0.75	0.83	0.87	0.82	1.66	0.69	0.90	0.95	0.93	0.10	-	-
19-Aug-91	+27	**	**	**	0.79	**	**	**	0.74	0.77	0.02	0.89	0.89	0.93	1.00	0.89	0.75	0.97	0.88	0.90	0.02	-	-
26-Aug-91	+28	**	**	**	0.79	**	**	**	0.67	0.73	0.04	0.89	0.67	1.21	0.97	1.02	0.95	1.07	0.80	0.95	0.05	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead;

the weeks when the median values of the infected and uninfected were significantly different are printed in bold faces.

Appendix Table 10.4.17
Serum Glutamate Dehydrogenase Activity (U/l) of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
14-Jan-91	-4	7	7	3	5	6	3	9	6	5.7	0.7	6	5	5	7	8	7	7	2	5.8	0.6	30.0	0.8785
21-Jan-91	-3	6	7	5	5	6	7	8	7	6.2	0.4	7	4	8	6	7	7	6	6	6.3	0.4	-	-
28-Jan-91	-2	7	6	4	4	8	9	10	4	6.4	0.8	5	5	7	5	6	6	3	7	5.7	0.4	-	-
04-Feb-91	-1	8	7	3	4	8	4	8	4	5.7	0.8	4	6	7	7	7	6	7	7	6.2	0.4	-	-
11-Feb-91	0	9	3	5	5	7	9	9	4	6.4	0.9	2	5	8	4	7	4	5	9	5.4	0.8	25.0	0.5054
18-Feb-91	+1	6	7	7	7	6	10	10	5	7.1	0.6	7	5	7	7	6	7	6	7	6.6	0.3	27.0	0.6454
25-Feb-91	**+2**	16	2	5	9	9	16	13	4	9.4	1.8	4	3	7	4	3	5	2	4	3.9	0.6	12.5	0.0379
04-Mar-91	**+3**	11	11	21	18	19	13	22	14	16.1	1.5	5	5	5	3	1	5	2	7	4.1	0.6	0.0	0.0002
11-Mar-91	**+4**	13	22	18	9	9	19	10	17	14.6	1.6	7	8	2	2	4	2	3	9	4.5	1.0	1.0	0.0003
18-Mar-91	**+5**	80	55	57	24	27	16	22	27	38.5	7.5	5	4	4	6	6	4	5	6	4.8	0.3	-	-
25-Mar-91	**+6**	80	64	84	42	69	32	13	35	52.4	8.5	4	5	3	6	5	7	5	6	5.1	0.4	-	-
01-Apr-91	**+7**	88	86	69	44	68	73	37	18	60.2	8.2	7	5	3	7	6	6	6	5	5.7	0.5	-	-
08-Apr-91	**+8**	164	146	329	73	146	119	91	91	144.9	26.7	5	5	5	3	5	5	5	6	5.1	0.3	0.0	0.0002
15-Apr-91	**+9**	164	173	356	183	123	146	91	228	183.0	26.7	6	7	6	6	5	5	6	5	5.8	0.3	-	-
22-Apr-91	**+10**	292	164	400	146	191	164	164	164	210.7	29.5	9	8	8	5	2	6	9	7	6.7	0.8	-	-
29-Apr-91	**+11**	365	201	383	274	319	292	73	219	265.8	33.3	9	9	6	8	4	4	7	7	6.8	0.7	-	-
06-May-91	**+12**	265	64	256	55	91	155	64	91	130.0	28.5	6	8	7	5	5	7	5	5	6.3	0.4	0.0	0.0002
13-May-91	**+13**	219	119	119	119	64	119	40	43	105.1	19.1	7	4	11	9	7	7	8	8	7.7	0.7	-	-
20-May-91	**+14**	155	365	110	110	64	123	110	37	134.0	33.1	8	4	6	10	13	9	6	6	7.7	0.9	-	-
27-May-91	**+15**	110	**	73	110	40	73	164	46	87.9	15.2	5	9	5	9	7	6	9	7	7.4	0.5	-	-
03-Jun-91	**+16**	100	**	137	64	37	**	73	64	79.1	13.0	9	7	6	11	9	7	7	7	8.1	0.5	0.0	0.0007
10-Jun-91	**+17**	119	**	82	82	82	**	18	64	74.5	12.2	4	7	7	9	9	13	4	4	6.9	1.1	-	-
17-Jun-91	**+18**	110	**	137	164	64	**	46	46	94.3	18.7	4	9	8	8	9	9	8	8	7.9	0.6	-	-
24-Jun-91	**+19**	9	**	**	119	**	**	82	46	63.9	20.4	8	4	6	9	7	4	6	9	6.8	0.7	-	-
01-Jul-91	**+20**	18	**	**	247	**	**	100	37	100.5	44.9	9	7	5	8	6	6	6	8	7.1	0.4	0.0	0.0040
08-Jul-91	**+21**	9	**	**	18	**	**	37	82	36.5	14.1	8	8	4	7	9	4	9	8	7.1	0.7	-	-
15-Jul-91	**+22**	46	**	**	82	**	**	46	110	70.7	13.5	9	10	8	9	10	9	9	6	8.8	0.4	-	-
22-Jul-91	**+23**	55	**	**	76	**	**	46	109	71.5	12.2	11	9	8	9	10	9	9	8	9.2	0.3	-	-
29-Jul-91	**+24**	46	**	**	46	**	**	27	109	56.7	15.6	10	9	9	9	10	9	9	8	9.1	0.2	0.0	0.0040
05-Aug-91	**+25**	46	**	**	46	**	**	26	73	47.4	8.4	6	6	6	8	10	9	4	4	6.7	0.8	-	-
12-Aug-91	**+26**	27	**	**	64	**	**	9	73	43.3	13.1	9	8	8	6	8	11	4	4	7.2	0.8	-	-
19-Aug-91	**+27**	**	**	**	73	**	**	**	60	66.5	4.6	7	9	8	8	8	9	4	8	7.7	0.5	-	-
26-Aug-91	**+28**	**	**	**	22	**	**	**	73	47.5	18.1	8	7	7	5	9	4	6	9	7.0	0.6	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead;

the weeks when the median values of infected and uninfected groups were significantly different are printed in bold faces.

Appendix Table 10.4.18
Serum Gamma Glutamyl Transpeptidase Activity (U/l) of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	U	P
14-Jan-91	-4	13	16	6	7	12	15	7	16	11.5	1.4	9	10	10	11	13	14	12	12	11.3	0.5	28.5	0.7209
21-Jan-91	-3	10	13	10	12	12	12	10	14	11.5	0.5	11	14	13	12	14	12	15	11	12.7	0.5	-	-
28-Jan-91	-2	10	14	9	9	15	13	8	16	11.6	1.0	13	16	14	14	14	8	16	8	12.8	1.0	-	-
04-Feb-91	-1	15	12	10	14	11	13	11	14	12.4	0.6	14	15	13	14	13	9	13	10	12.6	0.6	-	-
11-Feb-91	0	13	14	10	12	12	13	9	16	12.4	0.7	11	15	15	15	14	9	13	12	13.2	0.7	26.0	0.5737
18-Feb-91	+1	13	14	14	16	13	13	9	16	13.5	0.7	12	14	16	14	15	9	14	10	13.2	0.8	30.5	0.8785
25-Feb-91	+2	14	10	16	16	14	15	10	16	14.0	0.8	13	15	14	13	13	7	15	10	12.4	0.9	19.0	0.1949
04-Mar-91	+3	17	10	19	17	14	22	10	14	15.4	1.3	16	16	13	15	14	9	14	8	13.2	1.0	20.0	0.2345
11-Mar-91	+4	15	18	12	15	15	24	11	17	15.8	1.4	14	16	10	15	15	10	15	10	13.3	0.8	17.5	0.1304
18-Mar-91	+5	17	19	24	17	15	25	10	17	18.2	1.6	15	15	10	16	17	10	19	12	14.3	1.0	16.0	0.1049
25-Mar-91	+6	18	15	23	17	15	24	12	18	17.7	1.4	12	15	19	17	12	12	17	16	15.1	1.0	19.5	0.1949
01-Apr-91	+7	27	12	10	15	17	32	15	20	18.5	2.5	13	16	14	17	13	10	16	9	13.7	0.9	19.0	0.1949
08-Apr-91	**+8**	29	20	16	19	19	34	16	24	22.0	2.1	14	16	14	20	12	10	14	20	15.0	1.1	10.0	0.0207
15-Apr-91	**+9**	37	37	43	23	34	41	17	30	32.7	2.9	14	15	16	20	15	12	12	14	14.5	0.8	-	-
22-Apr-91	**+10**	37	47	54	27	50	52	22	30	39.9	4.2	12	14	15	19	15	12	13	9	13.5	1.0	-	-
29-Apr-91	**+11**	69	75	110	46	87	110	35	75	76.0	8.9	16	13	10	16	14	12	16	10	13.3	0.8	-	-
06-May-91	**+12**	110	104	139	72	64	116	52	75	91.5	9.9	15	12	19	22	17	12	14	12	15.4	1.2	0.0	0.0002
13-May-91	**+13**	110	104	139	73	75	120	64	76	95.2	8.9	14	17	17	18	13	11	16	14	15.1	0.9	-	-
20-May-91	**+14**	110	93	110	81	52	127	64	80	89.6	8.4	15	18	15	17	12	12	16	12	14.7	0.8	-	-
27-May-91	**+15**	116	**	93	93	69	129	64	81	92.1	8.3	7	13	16	17	15	7	17	14	13.4	1.4	-	-
03-Jun-91	**+16**	110	**	116	87	69	**	64	81	87.8	7.9	14	18	16	18	9	12	14	14	14.4	1.0	0.0	0.0007
10-Jun-91	**+17**	64	**	69	58	41	**	38	58	54.6	4.7	12	16	13	17	12	12	14	12	13.4	0.7	-	-
17-Jun-91	**+18**	64	**	**	75	**	**	41	75	42.4	13.1	14	15	10	14	13	10	15	13	13.0	0.6	-	-
24-Jun-91	**+19**	41	**	**	75	**	**	41	58	53.6	7.2	19	13	13	19	10	12	13	15	14.3	1.0	-	-
01-Jul-91	**+20**	41	**	**	69	**	**	35	42	46.6	6.7	15	12	10	19	12	10	14	14	13.2	0.9	0.0	0.0040
08-Jul-91	**+21**	69	**	**	75	**	**	52	122	79.6	12.8	15	15	13	16	14	12	15	15	14.3	0.5	-	-
15-Jul-91	**+22**	53	**	**	64	**	**	46	87	62.5	7.7	15	14	13	14	13	12	13	14	13.4	0.3	-	-
22-Jul-91	**+23**	52	**	**	82	**	**	46	122	75.4	14.9	16	16	13	15	12	10	16	15	14.2	0.7	-	-
29-Jul-91	**+24**	64	**	**	87	**	**	46	104	75.3	11.0	14	15	12	15	12	11	15	14	13.4	0.5	0.0	0.0040
05-Aug-91	**+25**	52	**	**	110	**	**	46	98	76.7	13.9	15	14	13	14	13	12	14	14	13.6	0.3	-	-
12-Aug-91	**+26**	35	**	**	75	**	**	41	81	57.9	10.2	15	15	14	16	12	12	14	12	13.7	0.5	-	-
19-Aug-91	**+27**	**	**	**	41	**	**	**	75	57.9	12.3	15	16	12	15	13	12	14	14	13.9	0.5	-	-
26-Aug-91	**+28**	**	**	**	41	**	**	**	75	57.9	12.3	15	15	12	14	13	13	14	13	13.7	0.4	-	-

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; ** =animals dead;

the weeks when the median values of infected and uninfected groups were significantly different are printed in bold faces.

Appendix Table 10.4.19
Serum Mineral Concentrations of Buffaloes
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
		81	83	85	87	89	92	94	96	Mean	SE	82	84	86	88	90	91	93	95	Mean	SE	t	P
CALCIUM (mmol/l)																							
14-Jan-91	-4	2.63	2.29	2.21	2.55	2.53	2.24	2.44	2.14	2.38	0.06	2.36	2.78	2.28	2.29	2.35	2.48	2.21	2.28	2.38	0.06	0.00	1.0000
11-Feb-91	0	2.70	2.32	3.10	2.77	2.29	2.89	3.14	3.04	2.78	0.11	2.43	2.79	2.72	3.32	2.82	2.97	3.57	2.73	2.92	0.12	0.79	0.4429
11-Mar-91	+4	3.67	1.53	2.43	3.10	2.31	2.28	2.18	2.19	2.46	0.21	2.93	2.35	2.19	2.38	2.01	2.58	2.58	2.61	2.45	0.09	0.03	0.9765
08-Apr-91	+8	2.26	2.48	2.08	2.82	2.25	2.25	2.19	2.14	2.31	0.08	2.19	2.16	2.28	2.51	2.25	1.90	2.84	2.48	2.33	0.09	0.13	0.8951
06-May-91	+12	2.19	2.12	2.01	2.50	2.17	3.17	2.20	2.22	2.32	0.12	2.54	2.95	2.79	2.64	2.38	2.00	3.22	2.48	2.63	0.12	1.63	0.1247
03-Jun-91	+16	1.28	**	2.50	2.22	2.16	**	2.07	2.33	2.09	0.16	2.13	3.48	1.99	2.17	2.22	2.41	2.28	2.22	2.36	0.15	1.11	0.2900
01-Jul-91	+20	1.67	**	**	1.86	**	**	1.94	2.00	1.87	0.06	2.01	2.40	2.20	2.25	2.09	2.25	2.08	2.28	2.20	0.04	4.04	0.0024
29-Jul-91	+24	1.89	**	**	1.87	**	**	2.22	2.34	2.08	0.10	2.28	2.41	2.26	2.29	2.55	2.54	2.21	2.57	2.39	0.05	2.82	0.0182
26-Aug-91	+28	**	**	**	2.09	**	**	**	1.99	2.04	0.04	2.18	2.82	2.39	2.35	2.47	2.65	2.60	2.25	2.46	0.07	2.64	0.0297
POTASSIUM (mmol/l)																							
14-Jan-91	-4	4.34	4.85	3.97	4.34	3.57	4.47	4.08	4.17	4.22	0.12	4.47	4.38	4.08	4.08	5.23	5.11	4.21	5.11	4.58	0.16	1.50	0.1558
11-Feb-91	0	4.60	3.57	4.08	4.60	4.85	3.83	4.08	4.08	4.21	0.14	4.34	4.34	3.57	4.60	3.83	3.57	4.08	3.83	4.02	0.13	0.94	0.3654
11-Mar-91	+4	4.21	3.83	3.57	4.72	4.60	4.72	4.85	4.60	4.39	0.15	3.96	4.21	4.08	3.83	4.47	3.96	4.85	4.72	4.26	0.13	0.60	0.5589
08-Apr-91	+8	3.96	4.34	4.08	4.08	3.70	4.21	4.34	4.34	4.13	0.07	4.47	3.57	3.83	4.34	3.70	4.47	3.06	4.85	4.04	0.20	0.42	0.6782
06-May-91	+12	4.85	4.85	3.57	4.09	3.57	4.21	4.21	4.21	4.20	0.16	4.85	3.57	3.57	4.21	3.57	4.85	3.57	4.85	4.13	0.21	0.23	0.8213
03-Jun-91	+16	4.72	**	4.47	4.34	4.08	**	4.08	3.96	4.28	0.11	4.85	4.98	4.60	4.34	4.39	5.23	4.34	4.60	4.67	0.11	2.33	0.0383
01-Jul-91	+20	3.86	**	**	4.09	**	**	4.09	3.83	3.97	0.06	3.86	4.08	4.55	4.55	4.09	6.26	4.47	3.51	4.42	0.27	1.06	0.3123
29-Jul-91	+24	3.67	**	**	4.21	**	**	4.85	4.21	4.24	0.21	5.23	4.08	4.21	4.72	4.21	5.11	3.45	4.47	4.44	0.19	0.65	0.5324
26-Aug-91	+28	**	**	**	4.12	**	**	**	4.18	4.15	0.02	4.85	4.47	4.08	4.47	4.08	5.36	4.21	4.47	4.50	0.14	1.19	0.2666
SODIUM (mmol/l)																							
14-Jan-91	-4	102	98	93	100	104	111	102	111	102.6	2.03	96	93	96	102	98	117	104	117	102.9	3.17	0.06	0.9506
11-Feb-91	0	136	117	113	115	152	141	128	136	129.8	4.61	106	106	117	136	141	148	143	133	128.9	5.48	0.13	0.8987
11-Mar-91	+4	117	143	113	122	143	143	117	141	130.1	4.60	111	113	109	141	115	128	113	128	119.8	3.79	1.61	0.1295
08-Apr-91	+8	109	93	109	111	117	120	117	124	112.5	3.12	113	93	117	124	115	115	124	115	114.5	3.20	0.42	0.6830
06-May-91	+12	124	124	116	124	67	93	109	124	110.1	6.75	146	124	124	124	78	109	109	124	117.1	6.43	0.71	0.4895
03-Jun-91	+16	113	**	109	116	109	**	96	93	105.7	3.49	106	111	106	106	109	109	96	100	105.4	1.67	0.16	0.8739
01-Jul-91	+20	115	**	**	115	**	**	108	103	110.3	2.67	108	125	117	107	117	107	113	135	116.1	3.30	1.17	0.2699
29-Jul-91	+24	117	**	**	102	**	**	101	104	106.1	3.32	128	106	100	109	106	109	109	117	110.6	2.83	0.96	0.3605
26-Aug-91	+28	**	**	**	106	**	**	**	103	104.7	1.23	117	124	102	109	115	122	111	111	113.8	2.36	1.87	0.0983
MAGNESIUM (mmol/l)																							
14-Jan-91	-4	0.88	0.96	0.79	0.82	1.02	0.91	0.75	0.96	0.89	0.03	0.88	0.93	0.83	0.96	0.76	0.96	0.85	0.86	0.88	0.02	0.28	0.7856
11-Feb-91	0	0.76	0.55	0.64	0.83	0.80	0.86	0.75	0.89	0.76	0.04	0.58	0.85	0.76	0.82	0.75	0.92	0.77	1.03	0.81	0.04	0.81	0.4330
11-Mar-91	+4	2.05	0.61	0.66	0.83	0.62	0.70	0.77	0.91	0.89	0.16	1.02	1.13	0.91	0.91	0.78	0.89	0.89	0.79	0.92	0.04	0.12	0.9046
08-Apr-91	+8	0.69	0.57	0.99	0.64	0.62	0.69	0.69	0.59	0.69	0.04	0.73	0.73	0.63	0.73	0.71	0.70	0.45	0.68	0.67	0.03	0.26	0.7979
06-May-91	+12	0.28	0.43	0.47	0.55	0.57	0.77	0.60	0.49	0.52	0.05	0.60	0.69	0.72	0.42	0.97	0.71	0.79	0.67	0.70	0.05	2.36	0.0333
03-Jun-91	+16	0.55	**	0.64	0.81	1.00	**	0.90	0.82	0.79	0.06	0.85	0.82	0.99	0.65	1.02	0.80	0.74	0.84	0.84	0.04	0.68	0.5088
01-Jul-91	+20	0.57	**	**	0.54	**	**	0.79	0.76	0.67	0.06	0.64	0.78	0.69	0.62	0.75	0.56	0.56	0.86	0.68	0.04	0.14	0.8901
29-Jul-91	+24	0.48	**	**	0.55	**	**	0.75	0.89	0.67	0.08	0.65	0.84	1.62	0.48	0.85	0.80	0.66	0.63	0.82	0.12	0.79	0.4496
26-Aug-91	+28	**	**	**	0.50	**	**	**	0.59	0.55	0.03	0.65	0.74	0.98	0.68	0.89	0.77	0.75	0.78	0.78	0.04	2.87	0.0207
INORGANIC PHOSPHORUS (mmol/l)																							
14-Jan-91	-4	2.45	2.76	1.80	1.94	1.40	2.17	2.20	1.80	2.07	0.14	2.68	2.45	1.39	2.26	2.37	2.48	1.89	2.17	2.21	0.13	0.70	0.4928
11-Feb-91	0	2.38	2.70	2.39	2.92	2.72	2.42	2.48	2.44	2.56	0.07	2.78	2.42	2.24	2.60	2.38	2.52	2.30	2.56	2.47	0.06	0.87	0.4007
11-Mar-91	+4	1.19	1.35	1.19	1.46	1.36	1.21	1.24	1.22	1.28	0.03	1.39	1.21	1.12	1.30	1.19	1.26	1.15	1.28	1.24	0.03	0.85	0.4094
08-Apr-91	+8	2.69	2.22	1.69	1.85	1.61	1.97	1.65	1.25	1.87	0.14	1.99	1.96	1.82	1.50	1.50	1.66	1.79	1.57	1.72	0.06	0.84	0.4143
06-May-91	+12	1.67	1.73	1.82	1.78	1.93	1.88	1.61	1.22	1.71	0.07	1.84	1.56	1.53	1.95	1.67	2.00	1.70	1.01	1.66	0.10	0.35	0.7313
03-Jun-91	+16	1.50	**	2.41	2.53	1.60	**	1.49	1.39	1.82	0.19	2.52	1.97	1.78	1.22	2.13	2.05	1.49	2.59	1.97	0.16	0.57	0.5820
01-Jul-91	+20	1.75	**	**	1.83	**	**	1.67	1.75	1.75	0.03	2.25	2.14	2.48	2.44	1.38	2.25	2.25	2.44	2.20	0.12	2.48	0.0323
29-Jul-91	+24	1.66	**	**	1.20	**	**	1.14	1.31	1.33	0.10	2.14	1.90	1.70	1.81	1.90	1.88	2.36	1.26	1.87	0.11	3.12	0.0108
26-Aug-91	+28	**	**	**	1.27	**	**	**	1.14	1.21	0.05	1.92	1.84	1.37	1.31	2.07	1.49	1.68	1.54	1.65	0.09	2.32	0.0486
COPPER (µmol/l)																							
14-Jan-91	-4	10.0	23.9	9.4	13.2	11.5	9.8	24.1	16.3	14.8	2.02	20.7	23.9	14.1	22.6	13.5	15.6	13.2	15.4	17.4	1.44	0.98	0.3432
11-Feb-91	0	24.6	9.9	8.4	12.7	12.4	10.2	14.7	18.1	13.9	1.76	11.1	9.0	12.4	19.3	11.1	23.3	12.7	13.8	14.1	1.58	0.08	0.9341
11-Mar-91	-4	11.2	21.4	22.3	19.0	14.0	20.5	10.9	23.0	17.8	1.66	16.7	11.4	21.4	20.0	15.1	20.5	11.2	18.9	16.9	1.33	0.39	0.7011
08-Apr-91	+8	24.6	12.0	19.3	16.3	13.2	12.0	19.8	16.3	16.7	1.45	18.0	17.8	16.0	19.8	16.5	11.8	11.0	15.8	15.8	1.00	0.45	0.6597
06-May-91	+12	13.9	10.1	21.0	12.7	17.5	16.5	17.5	11.7	15.1	1.20	24.2	16.9	14.7	17.8	10.3	12.4	20.6	15.1	16.5	1.47	0.68	0.5052
03-Jun-91	+16	10.5	**	17.2	22.8	16.0	**	19.1	9.5	15.9	1.90	14.5	18.3	17.9	12.6	13.9	14.2	22.2	25.9	17.4	1.53	0.61	0.5541
01-Jul-91	+20	9.2	**	**	12.9	**	**	19.9	20.2	15.5	2.32	10.8	12.3	9.9	14.9	14.4	22.1	16.2	11.7	14.1	1.29	0.56	0.5878
29-Jul-91	+24	14.4	**	**	12.5	**	**	13.3	18.8	14.7	1.22	11.9	19.8	12.5	12.5	10.4	16.4	18.3	15.4	14.6	1.11	0.05	0.9602
26-Aug-91	+28	**	**	**	12.9	**	**	**	13.9	13.4	0.36	18.0	18.3	12.8	9.4	10.3	18.4	22.0	22.0	16.4	1.64	0.82	0.4375

Statistics =results of unpaired t-test; t =t value; P =two-tailed P value (probability); ** =animals dead; the weeks when the means of infected and uninfected groupswere significantly different are printed in bold faces.

Appendix Table 20
Results of Agar Gel Diffusion Assay of the Sera of Buffaloes Calves
Experiment 4: Infection of buffaloes with high dose of *Fasciola gigantica* metacercariae

		Infected Animals (Group A)								Uninfected Animals (Group B)							
Date	Week	81	83	85	87	89	92	94	96	82	84	86	88	90	91	93	95
31-Dec-90	-6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
07-Jan-91	-5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14-Jan-91	-4	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-
21-Jan-91	-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28-Jan-91	-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04-Feb-91	-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11-Feb-91	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18-Feb-91	+1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25-Feb-91	+2	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
04-Mar-91	+3	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-
11-Mar-91	+4	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-
18-Mar-91	+5	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-
25-Mar-91	+6	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-
01-Apr-91	+7	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-
08-Apr-91	+8	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-
15-Apr-91	+9	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-
22-Apr-91	+10	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-
29-Apr-91	+11	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-
06-May-91	+12	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
13-May-91	+13	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
20-May-91	+14	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
27-May-91	+15	+	**	+	+	+	+	+	+	-	-	-	-	-	-	-	-
03-Jun-91	+16	+	**	+	+	+	**	-	+	-	-	-	-	-	-	-	-
10-Jun-91	+17	+	**	+	+	+	**	+	+	-	-	-	-	-	-	-	-
17-Jun-91	+18	+	**	+	+	+	**	+	+	-	-	-	-	-	-	-	-
24-Jun-91	+19	+	**	**	+	**	**	+	+	-	-	-	-	-	-	-	-
01-Jul-91	+20	-	**	**	+	**	**	-	+	-	-	-	-	-	-	-	-
08-Jul-91	+21	+	**	**	+	**	**	+	+	-	-	-	-	-	-	-	-
15-Jul-91	+22	-	**	**	+	**	**	-	+	-	-	-	-	-	-	-	-
22-Jul-91	+23	-	**	**	-	**	**	+	+	-	-	-	-	-	-	-	-
29-Jul-91	+24	+	**	**	-	**	**	+	+	-	-	-	-	-	-	-	-
05-Aug-91	+25	+	**	**	+	**	**	-	-	-	-	-	-	-	-	-	-
12-Aug-91	+26	-	**	**	-	**	**	+	+	-	-	-	-	-	-	-	-
19-Aug-91	+27	**	**	**	+	**	**	**	+	-	-	-	-	-	-	-	-
26-Aug-91	+28	**	**	**	+	**	**	**	-	-	-	-	-	-	-	-	-

+ = positive for precipitin line; - = negative for precipitin line; ** =animals dead.

Appendix Table 8.5.1
Liveweights (kg) of the individual animals
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
09-Oct-91	-5	85	97	79	66	102	89	80	93	86.0	3.7	80	92	78	57	95	98	79	95	84.3	4.5	30.0	0.8785
16-Oct-91	-4	89	102	83	67	105	86	81	94	88.4	4.0	82	94	82	59	99	101	80	98	86.9	4.7	-	-
23-Oct-91	-3	87	105	84	69	106	90	84	99	90.5	4.1	84	99	85	61	103	104	82	102	90.0	4.9	-	-
30-Oct-91	-2	90	109	85	65	111	93	85	99	92.1	4.9	85	99	90	60	105	108	83	103	91.6	5.2	-	-
06-Nov-91	-1	94	113	92	76	116	100	91	101	97.9	4.2	89	107	93	62	108	112	83	106	95.0	5.6	-	-
13-Nov-91	0	98	114	90	75	118	98	93	107	99.1	4.6	93	109	99	61	107	112	87	117	98.1	6.0	31.0	0.9591
20-Nov-91	+1	100	119	96	81	120	100	95	110	102.6	4.3	95	110	96	62	110	117	87	113	98.8	6.0	-	-
27-Nov-91	+2	102	123	97	84	122	102	100	112	105.3	4.3	99	115	101	63	113	118	91	121	102.6	6.3	-	-
04-Dec-91	+3	104	124	94	89	119	103	102	112	105.9	3.9	96	119	98	65	113	124	91	121	103.4	6.6	-	-
11-Dec-91	+4	109	128	99	96	123	109	109	118	111.4	3.7	103	125	99	64	116	125	96	126	106.8	7.0	-	-
18-Dec-91	+5	113	130	98	98	132	113	110	124	114.8	4.3	108	127	105	69	123	130	100	132	111.8	7.0	31.0	0.9591
25-Dec-91	+6	118	138	103	102	134	118	111	127	118.9	4.4	109	133	103	70	122	134	105	135	113.9	7.3	-	-
01-Jan-92	+7	123	140	105	104	132	118	110	129	120.1	4.4	112	133	107	71	124	139	108	140	116.8	7.5	-	-
08-Jan-92	+8	119	134	102	103	133	119	110	130	118.8	4.3	113	132	109	73	128	138	105	138	117.0	7.3	-	-
15-Jan-92	+9	126	142	108	112	135	125	116	136	125.0	4.1	111	139	111	75	131	143	111	149	121.3	8.0	-	-
22-Jan-92	+10	119	138	105	108	136	124	115	130	121.9	4.1	119	135	112	75	136	147	111	150	123.1	8.1	28.0	0.7209
29-Jan-92	+11	118	135	106	112	139	129	122	138	124.9	4.1	118	141	111	78	133	147	115	155	124.8	8.2	-	-
05-Feb-92	+12	117	128	103	108	134	130	119	134	121.6	3.9	123	140	112	80	132	145	115	150	124.6	7.5	-	-
12-Feb-92	+13	118	132	105	108	137	128	122	139	123.6	4.2	125	138	114	81	134	145	117	149	125.4	7.2	-	-
19-Feb-92	+14	120	136	107	109	135	132	122	141	125.3	4.2	128	139	116	80	139	150	118	152	127.8	7.8	-	-
26-Feb-92	+15	117	138	109	111	133	130	126	139	125.4	3.9	127	138	115	82	141	148	120	146	127.1	7.2	24.5	0.4418
04-Mar-92	+16	120	140	112	115	135	133	123	140	127.3	3.7	131	140	118	85	145	146	119	149	129.1	7.1	-	-
11-Mar-92	+17	119	145	113	117	135	136	122	145	129.0	4.2	130	145	120	84	149	150	120	152	131.3	7.7	-	-
18-Mar-92	+18	115	145	115	121	139	142	129	150	132.0	4.6	135	150	119	86	153	155	122	157	134.6	8.2	-	-
25-Mar-92	+19	118	145	117	119	140	146	130	148	132.9	4.5	138	148	122	87	151	155	122	158	135.1	7.9	-	-
01-Apr-92	+20	122	146	115	118	135	144	130	150	132.5	4.4	136	152	120	86	153	156	124	158	135.6	8.2	22.0	0.3282
08-Apr-92	+21	129	147	122	120	148	149	133	150	137.3	4.2	140	154	123	89	158	161	129	162	139.5	8.3	-	-
15-Apr-92	+22	134	149	126	124	151	151	135	153	140.4	4.0	145	155	125	93	162	165	132	169	143.3	8.5	-	-
22-Apr-92	+23	138	150	129	129	152	150	136	154	142.3	3.4	150	165	124	92	165	169	135	173	146.6	9.3	-	-
29-Apr-92	+24	124	145	130	128	150	151	134	157	139.9	4.1	147	163	126	91	162	172	136	170	145.9	9.1	-	-
06-May-92	+25	120	158	129	126	148	153	136	161	141.4	5.2	149	166	125	95	165	170	138	171	147.4	8.9	22.0	0.3282
13-May-92	+26	133	155	128	125	145	155	137	160	142.3	4.4	150	165	127	94	165	168	137	170	147.0	8.8	-	-
20-May-92	+27	125	156	123	129	147	151	134	158	140.4	4.7	151	168	130	96	165	171	139	172	149.0	8.8	-	-
27-May-92	+28	127	152	120	124	144	154	135	159	139.4	4.9	150	167	133	99	164	171	140	170	149.3	8.2	-	-
03-Jun-92	+29	127	149	128	129	140	142	140	159	139.3	3.7	148	168	131	102	163	171	139	170	149.0	8.0	-	-
10-Jun-92	+30	129	153	124	134	145	145	139	155	140.5	3.7	153	169	133	102	165	174	143	168	150.9	8.0	19.5	0.1949
17-Jun-92	**+31**	118	147	120	128	133	136	131	151	133.0	3.9	153	165	135	105	164	172	140	167	150.1	7.5	**13.0**	**0.0499**
24-Jun-92	**+32**	118	140	116	124	130	136	128	152	130.5	3.9	155	170	136	104	166	178	142	173	153.0	8.2	**11.5**	**0.0281**
01-Jul-92	+33	123	145	120	128	139	138	135	157	135.6	4.0	159	171	135	107	171	175	144	175	154.6	8.1	14.5	0.0650
08-Jul-92	**+34**	122	145	121	131	141	137	133	158	136.0	4.0	158	174	135	109	172	173	149	179	156.1	8.0	**13.5**	**0.0499**
15-Jul-92	+35	125	139	120	132	145	139	139	162	137.6	4.2	161	172	137	111	178	182	148	183	159.0	8.4	15.0	0.0830

Statistics = results of Mann-Whitney test; U = U statistic; P = two-tailed P (probability) value; - = analysis not done; the weeks when the median values of the groups were significantly different are printed in bold faces.

Appendix Table 8.5.2
Regression statistics of the liveweight of individual animals
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

Group	Buffalo No	n	Line of best fit (Y=a+bX)	r2	P	Liveweight gain (kg/week)
A	61	36	Y = 109.64+0.57X	0.457	< 0.001	0.572
	62	36	Y = 126.61+0.78X	0.612	< 0.001	0.781
	63	36	Y = 96.04+0.95X	0.776	< 0.001	0.949
	64	36	Y = 90.21+1.36X	0.869	< 0.001	1.355
	65	36	Y = 126.26+0.60X	0.502	< 0.001	0.601
	66	36	Y = 109.72+1.30X	0.684	< 0.001	1.296
	67	36	Y = 103.26+1.15X	0.845	< 0.001	1.149
	68	36	Y = 116.49+1.45X	0.880	< 0.001	1.447
	Mean	36	Y = 109.80+1.02X	0.785	< 0.001	1.018
B	71	36	Y = 97.17+1.64X	0.972	< 0.001	1.639
	72	36	Y = 116.39+1.76X	0.953	< 0.001	1.762
	73	36	Y = 98.09+1.16X	0.981	< 0.001	1.160
	74	36	Y = 61.27+1.37X	0.990	< 0.001	1.366
	75	36	Y = 111.90+1.62X	0.957	< 0.001	1.619
	76	36	Y = 121.50+1.78X	0.947	< 0.001	1.781
	77	36	Y = 91.69+1.71X	0.970	< 0.001	1.705
	78	36	Y = 124.11+1.70X	0.903	< 0.001	1.701
	Mean	36	Y = 102.79+1.67X	0.980	< 0.001	1.666

Appendix Table 8.5.3
Comparisions of the slopes (b) of regression lines of liveweights against WPI
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

Comparision	d.f.	Slopes (b)	F value	t value	Probability (P)
Buffalo 61 vs 71	1	0.57 vs 1.64	125.50	11.203	< 0.001
Buffalo 62 vs 72	1	0.78 vs 1.76	60.71	7.792	< 0.001
Buffalo 63 vs 73	1	0.95 vs 1.16	5.30	2.302	0.024
Buffalo 64 vs 74	1	1.36 vs 1.37	0.01	0.100	> 0.05
Buffalo 65 vs 75	1	0.60 vs 1.62	112.83	10.622	< 0.001
Buffalo 66 vs 76	1	1.30 vs 1.78	8.43	2.904	0.005
Buffalo 67 vs 77	1	1.15 vs 1.71	31.50	5.613	< 0.001
Buffalo 68 vs 78	1	1.45 vs 1.70	3.70	1.924	0.059
Group A vs B	14	1.02 vs 1.67	39.90	6.317	< 0.001

Appendix Table 8.5.4
Fasciola gigantica faecal egg counts (EPG) of Buffalo calves
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
08-Oct-91	-5	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
15-Oct-91	-4	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
22-Oct-91	-3	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
29-Oct-91	-2	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
05-Nov-91	-1	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
12-Nov-91	0	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
19-Nov-91	+1	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
26-Nov-91	+2	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
03-Dec-91	+3	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
10-Dec-91	+4	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
17-Dec-91	+5	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
24-Dec-91	+6	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
31-Dec-91	+7	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
07-Jan-92	+8	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
14-Jan-92	+9	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
21-Jan-92	+10	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
28-Jan-92	+11	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
04-Feb-92	+12	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
11-Feb-92	+13	2	12	0	0	0	1	0	0	1.9	1.4	0	0	0	0	0	0	0	0	0.0	0.0	-	-
18-Feb-92	+14	8	2	1	1	2	1	0	0	1.9	0.9	0	0	0	0	0	0	0	0	0.0	0.0	-	-
25-Feb-92	+15	4	21	5	2	6	19	1	10	8.5	2.5	0	0	0	0	0	0	0	0	0.0	0.0	-	-
03-Mar-92	+16	10	8	3	1	0	24	3	3	6.5	2.6	0	0	0	0	0	0	0	0	0.0	0.0	-	-
10-Mar-92	+17	88	3	0	0	0	3	0	5	12.4	10.2	0	0	0	0	0	0	0	0	0.0	0.0	-	-
17-Mar-92	+18	31	1	4	1	25	11	0	3	9.6	4.0	0	0	0	0	0	0	0	0	0.0	0.0	-	-
24-Mar-92	+19	7	0	0	5	47	5	3	4	8.8	5.1	0	0	0	0	0	0	0	0	0.0	0.0	-	-
31-Mar-92	+20	4	10	3	7	3	26	0	1	6.7	2.8	0	0	0	0	0	0	0	0	0.0	0.0	-	-
07-Apr-92	+21	4	0	3	0	14	8	0	12	5.0	1.9	0	0	0	0	0	0	0	0	0.0	0.0	-	-
14-Apr-92	+22	36	27	0	1	4	8	0	3	9.9	4.6	0	0	0	0	0	0	0	0	0.0	0.0	-	-
21-Apr-92	+23	26	48	1	1	1	3	0	3	10.4	5.8	0	0	0	0	0	0	0	0	0.0	0.0	-	-
28-Apr-92	+24	28	16	0	3	3	0	1	3	6.5	3.3	0	0	0	0	0	0	0	0	0.0	0.0	-	-
05-May-92	+25	22	5	5	8	3	4	1	13	7.6	2.3	0	0	0	0	0	0	0	0	0.0	0.0	-	-
12-May-92	+26	56	7	10	1	1	1	0	0	9.6	6.3	0	0	0	0	0	0	0	0	0.0	0.0	-	-
19-May-92	+27	33	17	7	8	1	18	1	0	10.6	3.7	0	0	0	0	0	0	0	0	0.0	0.0	-	-
26-May-92	+28	36	10	35	0	8	7	4	4	13.0	4.8	0	0	0	0	0	0	0	0	0.0	0.0	-	-
02-Jun-92	+29	75	38	18	12	21	8	1	14	23.4	7.8	0	0	0	0	0	0	0	0	0.0	0.0	-	-
09-Jun-92	+30	46	5	1	1	0	0	0	5	7.3	5.2	0	0	0	0	0	0	0	0	0.0	0.0	-	-
16-Jun-92	+31	18	8	7	5	21	5	0	3	8.3	2.4	0	0	0	0	0	0	0	0	0.0	0.0	-	-
23-Jun-92	+32	23	60	10	3	8	12	1	3	15.0	6.4	0	0	0	0	0	0	0	0	0.0	0.0	-	-
30-Jun-92	+33	22	14	3	1	21	0	0	29	11.2	3.8	0	0	0	0	0	0	0	0	0.0	0.0	-	-
07-Jul-92	+34	25	68	22	5	16	3	1	3	17.7	7.3	0	0	0	0	0	0	0	0	0.0	0.0	-	-
14-Jul-92	+35	33	78	18	4	14	12	2	9	21.2	8.2	0	0	0	0	0	0	0	0	0.0	0.0	-	-

Statistics = results of Mann-Whitney U test; U = U statistic; P = two-tailed P value (probability); - = analysis not done.

Appendix Table 8.5.5
Some Carcass Data of the Buffalo Calves
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

	Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	t	P
Carcass wt (kg)	125	139	120	132	145	139	139	162	137.6	4.25	161	172	137	111	178	182	148	183	159.0	8.43	2.27	0.040
Dressed carcass (kg) ..	45.2	51.6	46.0	50.8	54.3	52.4	51.6	61.0	51.61	1.63	63.7	71.0	65.7	51.2	73.5	74.2	66.8	73.2	67.41	2.53	5.25	<0.001
Killing out %	36.2	37.1	38.3	38.5	37.4	37.7	37.1	37.7	37.5	0.24	39.6	41.3	48.0	46.1	41.3	40.8	45.1	40.0	42.77	1.05	4.84	<0.001
Weight of liver (kg)	1.80	1.95	1.55	1.70	1.90	2.50	1.70	2.25	1.919	0.10	1.80	2.20	1.20	1.40	1.50	2.00	1.80	2.25	1.769	0.13	0.91	0.376
Liver wt. in proportion to carcass wt. (g/kg)...	14.4	14.0	12.9	12.9	13.1	18.0	12.2	13.9	13.93	0.59	11.2	12.8	8.8	12.6	8.4	11.0	12.2	12.3	11.15	0.56	3.42	0.004

Statistics = results of unpaired t test; t = t values; P = two-tailed P values (probability).

Appendix Table 8.5.6: RBC Counts ($\times 10^{12}$ cells/litre of blood) of Buffaloes
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	6.74	5.94	6.43	6.35	5.42	5.80	6.40	7.40	6.31	0.20	6.19	6.21	5.08	6.41	6.53	5.28	6.75	6.51	6.12	0.20	30.0	0.8785
14-Oct-91	-4	6.41	6.26	6.30	7.10	5.60	6.25	6.96	7.61	6.56	0.21	6.80	7.19	4.10	6.44	6.13	6.04	6.14	7.49	6.29	0.34	-	-
21-Oct-91	-3	6.91	6.70	6.70	7.34	6.49	6.64	7.08	7.46	6.92	0.12	7.49	7.34	5.10	6.50	7.23	5.76	7.40	8.00	6.85	0.33	-	-
28-Oct-91	-2	6.60	6.89	6.91	7.45	4.24	6.55	6.71	7.15	6.56	0.33	6.85	6.31	6.89	6.36	6.09	5.25	7.51	8.25	6.69	0.30	-	-
04-Nov-91	-1	6.48	6.74	6.95	6.90	4.41	7.54	6.71	7.23	6.62	0.31	6.85	6.98	7.10	6.79	6.06	6.25	7.00	8.65	6.96	0.26	-	-
11-Nov-91	0	6.27	6.87	6.32	6.57	4.77	7.13	5.82	6.87	6.33	0.25	6.92	6.80	7.22	6.47	5.87	5.20	6.27	8.42	6.64	0.32	26.5	0.5737
18-Nov-91	+1	6.82	6.97	6.63	6.53	5.22	7.02	6.88	6.80	6.61	0.19	6.53	7.00	7.00	7.10	6.83	5.25	7.12	7.47	6.79	0.22	-	-
25-Nov-91	+2	5.82	6.17	6.08	5.92	5.35	6.62	6.60	6.32	6.11	0.14	6.05	7.05	6.75	6.25	6.90	5.17	6.87	8.32	6.67	0.30	-	-
02-Dec-91	+3	5.50	6.15	5.73	5.95	5.68	6.63	6.70	6.63	6.12	0.16	5.73	6.17	6.37	5.97	7.35	5.37	6.68	8.65	6.54	0.35	-	-
09-Dec-91	+4	6.02	6.27	5.82	5.97	5.77	6.98	7.42	5.90	6.27	0.20	5.70	6.42	6.25	5.72	6.93	5.32	7.17	8.13	6.45	0.31	-	-
16-Dec-91	+5	6.43	6.60	6.20	6.20	6.13	6.60	6.70	5.87	6.34	0.10	6.50	6.70	6.70	6.10	6.90	5.60	7.20	8.00	6.71	0.24	19.0	0.1949
23-Dec-91	+6	7.18	7.02	6.57	7.20	6.07	6.60	8.20	6.30	6.89	0.22	6.23	6.82	6.45	6.23	6.98	5.78	7.42	6.07	6.50	0.18	-	-
30-Dec-91	+7	6.80	7.25	6.73	6.85	6.95	6.87	7.90	6.32	6.96	0.15	6.50	7.15	6.85	6.55	7.25	7.10	7.65	8.12	7.15	0.18	-	-
06-Jan-92	+8	7.08	7.13	6.73	6.65	6.83	6.52	7.68	6.67	6.91	0.12	6.40	6.72	7.28	6.72	6.75	6.22	7.85	8.85	7.10	0.29	-	-
13-Jan-92	+9	7.23	7.27	6.60	7.08	6.60	6.90	7.27	5.77	6.84	0.17	6.67	7.35	7.05	6.80	7.30	6.22	7.50	8.00	7.11	0.18	-	-
20-Jan-92	+10	7.10	7.03	6.67	6.62	6.35	6.20	6.67	6.23	6.61	0.11	6.60	6.95	7.45	6.28	6.98	6.30	7.40	7.95	6.99	0.19	21.0	0.2786
27-Jan-92	+11	7.38	6.80	6.68	6.68	6.78	6.03	6.25	6.02	6.58	0.15	7.02	7.22	7.15	7.40	7.25	6.13	7.10	7.25	7.06	0.13	-	-
03-Feb-92	+12	7.85	6.27	6.65	6.80	6.87	5.90	8.00	6.82	6.89	0.24	6.70	7.95	7.48	7.40	7.57	6.55	7.43	7.83	7.36	0.16	-	-
10-Feb-92	+13	7.87	5.30	7.25	7.70	6.83	6.30	7.77	6.75	6.97	0.29	6.75	7.10	7.05	7.18	7.02	6.48	7.28	7.90	7.10	0.14	-	-
17-Feb-92	+14	7.07	6.55	7.13	7.40	7.12	6.37	7.97	6.90	7.06	0.16	7.07	7.42	7.15	7.32	7.45	6.57	7.87	8.33	7.40	0.18	-	-
24-Feb-92	+15	6.52	5.70	6.78	7.25	6.38	6.05	8.10	6.35	6.64	0.25	7.03	7.10	7.17	7.20	8.00	6.27	7.45	8.60	7.35	0.23	16.0	0.1049
02-Mar-92	+16	5.92	5.53	6.70	7.40	6.77	6.18	7.32	6.53	6.54	0.21	6.40	8.30	7.47	6.35	7.60	6.50	7.50	8.92	7.38	0.31	-	-
09-Mar-92	+17	5.95	5.42	6.63	7.18	7.05	5.93	7.43	6.20	6.48	0.24	6.30	7.60	7.25	7.65	6.78	5.32	7.32	8.10	7.04	0.29	-	-
16-Mar-92	+18	4.98	4.70	6.43	7.15	6.32	5.95	7.15	6.37	6.13	0.30	6.63	8.00	6.98	6.88	6.70	6.10	7.33	7.20	6.98	0.19	-	-
23-Mar-92	+19	4.68	4.68	6.12	6.97	5.22	5.00	6.65	5.68	5.63	0.29	6.80	7.90	6.80	6.00	6.80	6.10	6.90	7.18	6.81	0.20	-	-
30-Mar-92	+20	4.40	4.23	6.12	6.60	5.73	5.75	7.50	5.55	5.74	0.36	6.20	6.50	6.07	6.15	6.05	5.70	6.40	7.75	6.35	0.20	19.0	0.1949
06-Apr-92	+21	4.83	4.57	6.07	6.35	5.13	5.75	7.80	6.55	5.88	0.35	6.45	5.95	6.90	6.30	6.58	5.45	7.05	7.40	6.51	0.21	-	-
13-Apr-92	+22	4.32	4.25	5.90	5.27	4.55	5.45	6.30	5.15	5.15	0.25	6.80	6.20	6.10	6.50	6.42	5.80	6.50	6.78	6.39	0.11	-	-
20-Apr-92	+23	4.78	4.23	6.10	5.55	4.85	5.47	7.75	5.13	5.48	0.36	6.10	6.02	6.40	5.93	6.50	5.70	6.07	6.20	6.11	0.08	-	-
27-Apr-92	+24	4.85	4.35	6.45	5.80	4.88	5.78	7.45	5.53	5.64	0.33	5.65	6.53	6.50	5.70	6.45	5.73	6.75	7.12	6.30	0.18	-	-
04-May-92	+25	4.67	4.85	6.10	5.08	5.30	5.57	7.02	5.80	5.55	0.25	5.65	6.50	5.75	6.48	7.05	5.15	6.75	7.38	6.34	0.25	14.0	0.0650
11-May-92	+26	5.28	5.45	6.75	6.10	5.25	5.92	7.40	6.45	6.08	0.25	5.85	6.02	5.87	7.38	6.80	5.93	6.83	7.50	6.52	0.23	-	-
18-May-92	+27	4.87	4.40	6.73	6.10	4.93	6.03	6.65	5.92	5.70	0.29	5.70	6.50	5.78	6.25	6.65	5.85	6.12	6.80	6.21	0.14	-	-
25-May-92	+28	4.73	4.30	6.83	6.52	5.10	6.50	7.50	6.08	5.95	0.37	5.98	6.50	6.82	6.67	7.00	6.43	5.82	7.07	6.95	0.18	-	-
01-Jun-92	+29	4.55	4.58	6.38	6.10	5.50	6.60	6.65	5.67	5.75	0.28	6.37	6.35	6.60	5.87	6.57	5.75	7.13	6.73	6.42	0.15	-	-
08-Jun-92	+30	4.60	4.48	6.10	6.50	5.05	6.62	7.53	5.97	5.86	0.35	6.65	6.77	6.30	5.83	6.70	6.50	7.53	6.97	6.93	0.18	15.0	0.0830
15-Jun-92	+31	4.30	4.62	6.10	6.55	5.53	7.00	7.10	6.83	6.00	0.36	6.15	6.55	6.48	6.13	6.87	5.98	7.85	7.40	7.18	0.25	-	-
22-Jun-92	+32	4.50	4.97	6.50	6.92	5.63	6.23	7.40	7.00	6.14	0.34	6.30	6.60	6.17	6.57	6.27	5.90	6.85	7.65	6.54	0.18	-	-
29-Jun-92	+33	5.45	5.40	6.40	6.30	5.80	5.90	7.47	6.55	6.16	0.22	6.37	6.30	7.35	6.33	6.08	5.80	7.45	6.62	6.54	0.19	-	-
06-Jul-92	+34	4.95	5.10	6.40	6.10	5.55	6.82	7.18	6.60	6.09	0.27	6.40	6.97	6.35	6.33	6.20	5.10	6.77	7.83	6.49	0.26	-	-
13-Jul-92	+35	5.68	4.68	6.52	6.31	5.52	6.67	7.18	6.75	6.56	0.40	7.07	7.08	6.33	6.53	7.45	5.50	7.08	7.68	6.84	0.23	30.0	0.8785

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done.

Appendix Table 8.5.7: Packed Cell Volumes (l/l) of Buffaloes
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	0.36	0.33	0.37	0.35	0.32	0.29	0.36	0.30	0.34	0.01	0.31	0.30	0.29	0.34	0.34	0.32	0.33	0.37	0.33	0.01	25.5	0.5054
14-Oct-91	-4	0.35	0.35	0.36	0.36	0.32	0.30	0.35	0.34	0.34	0.01	0.32	0.35	0.22	0.34	0.33	0.34	0.33	0.39	0.33	0.02	-	-
21-Oct-91	-3	0.34	0.35	0.36	0.35	0.31	0.28	0.35	0.35	0.34	0.01	0.34	0.33	0.27	0.33	0.32	0.34	0.33	0.40	0.33	0.01	-	-
28-Oct-91	-2	0.34	0.35	0.35	0.36	0.31	0.29	0.36	0.33	0.34	0.01	0.32	0.32	0.33	0.34	0.30	0.31	0.34	0.38	0.33	0.01	-	-
04-Nov-91	-1	0.32	0.30	0.34	0.33	0.30	0.33	0.34	0.35	0.33	0.01	0.33	0.34	0.35	0.33	0.30	0.31	0.31	0.38	0.33	0.01	-	-
11-Nov-91	0	0.35	0.34	0.33	0.34	0.32	0.32	0.32	0.33	0.33	0.00	0.30	0.30	0.35	0.33	0.31	0.35	0.33	0.39	0.33	0.01	31.0	0.9591
18-Nov-91	+1	0.37	0.32	0.34	0.31	0.30	0.33	0.36	0.33	0.33	0.01	0.32	0.33	0.34	0.33	0.32	0.30	0.32	0.34	0.33	0.00	-	-
25-Nov-91	+2	0.35	0.32	0.33	0.33	0.31	0.32	0.37	0.33	0.33	0.01	0.30	0.34	0.34	0.31	0.35	0.30	0.34	0.35	0.33	0.01	-	-
02-Dec-91	+3	0.32	0.32	0.33	0.34	0.32	0.31	0.36	0.34	0.33	0.01	0.29	0.30	0.31	0.30	0.36	0.32	0.34	0.38	0.33	0.01	-	-
09-Dec-91	+4	0.33	0.30	0.31	0.34	0.31	0.33	0.39	0.31	0.33	0.01	0.29	0.28	0.32	0.31	0.35	0.33	0.35	0.38	0.33	0.01	-	-
16-Dec-91	+5	0.31	0.33	0.31	0.34	0.36	0.30	0.34	0.35	0.33	0.01	0.31	0.31	0.33	0.32	0.35	0.32	0.37	0.37	0.34	0.01	28.0	0.7209
23-Dec-91	+6	0.38	0.35	0.34	0.36	0.34	0.30	0.41	0.31	0.35	0.01	0.33	0.33	0.32	0.32	0.34	0.35	0.38	0.36	0.34	0.01	-	-
30-Dec-91	+7	0.38	0.36	0.35	0.36	0.35	0.32	0.41	0.33	0.36	0.01	0.32	0.35	0.37	0.35	0.34	0.36	0.37	0.37	0.35	0.01	-	-
06-Jan-92	+8	0.35	0.33	0.34	0.35	0.35	0.29	0.30	0.33	0.33	0.01	0.31	0.33	0.36	0.34	0.33	0.36	0.33	0.38	0.34	0.01	-	-
13-Jan-92	+9	0.38	0.34	0.34	0.36	0.35	0.30	0.36	0.39	0.35	0.01	0.34	0.34	0.36	0.35	0.34	0.35	0.34	0.37	0.35	0.00	-	-
20-Jan-92	+10	0.39	0.33	0.34	0.35	0.35	0.29	0.39	0.33	0.35	0.01	0.33	0.34	0.37	0.35	0.35	0.37	0.36	0.38	0.36	0.01	24.5	0.4418
27-Jan-92	+11	0.36	0.34	0.37	0.35	0.35	0.29	0.37	0.30	0.34	0.01	0.34	0.33	0.38	0.38	0.37	0.36	0.35	0.37	0.36	0.01	-	-
03-Feb-92	+12	0.40	0.32	0.36	0.35	0.35	0.29	0.41	0.36	0.36	0.01	0.35	0.35	0.38	0.38	0.36	0.35	0.37	0.37	0.36	0.00	-	-
10-Feb-92	+13	0.36	0.31	0.38	0.38	0.34	0.30	0.40	0.33	0.35	0.01	0.34	0.33	0.36	0.38	0.34	0.36	0.36	0.36	0.35	0.01	-	-
17-Feb-92	+14	0.38	0.32	0.39	0.37	0.33	0.31	0.42	0.35	0.36	0.01	0.34	0.34	0.36	0.37	0.36	0.37	0.38	0.39	0.36	0.01	-	-
24-Feb-92	+15	0.33	0.29	0.34	0.38	0.31	0.30	0.40	0.32	0.33	0.01	0.33	0.35	0.38	0.38	0.40	0.34	0.35	0.40	0.37	0.01	14.0	0.0650
02-Mar-92	+16	0.31	0.29	0.36	0.37	0.34	0.30	0.39	0.34	0.34	0.01	0.32	0.39	0.37	0.35	0.37	0.37	0.38	0.42	0.37	0.01	-	-
09-Mar-92	+17	0.30	0.28	0.33	0.37	0.32	0.29	0.37	0.32	0.32	0.01	0.33	0.35	0.40	0.37	0.36	0.36	0.36	0.38	0.36	0.01	-	-
16-Mar-92	+18	0.27	0.26	0.33	0.34	0.28	0.28	0.34	0.31	0.30	0.01	0.32	0.33	0.35	0.35	0.33	0.33	0.34	0.33	0.34	0.00	-	-
23-Mar-92	+19	0.27	0.27	0.35	0.35	0.27	0.27	0.36	0.28	0.30	0.01	0.32	0.33	0.34	0.34	0.32	0.33	0.34	0.35	0.33	0.00	-	-
30-Mar-92	+20	0.27	0.25	0.32	0.32	0.27	0.29	0.36	0.32	0.30	0.01	0.30	0.33	0.31	0.33	0.32	0.34	0.33	0.36	0.33	0.01	15.0	0.0830
06-Apr-92	+21	0.29	0.27	0.34	0.32	0.26	0.27	0.38	0.33	0.31	0.01	0.30	0.32	0.32	0.33	0.33	0.35	0.33	0.35	0.33	0.01	-	-
13-Apr-92	+22	0.28	0.25	0.33	0.29	0.24	0.27	0.36	0.29	0.29	0.01	0.31	0.32	0.33	0.32	0.31	0.34	0.32	0.34	0.32	0.00	-	-
20-Apr-92	+23	0.29	0.28	0.33	0.30	0.26	0.26	0.37	0.27	0.30	0.01	0.30	0.32	0.32	0.30	0.33	0.32	0.33	0.33	0.32	0.00	-	-
27-Apr-92	+24	0.28	0.25	0.33	0.30	0.25	0.27	0.37	0.29	0.29	0.01	0.31	0.31	0.30	0.31	0.32	0.32	0.33	0.33	0.32	0.00	-	-
04-May-92	+25	0.27	0.26	0.35	0.30	0.28	0.29	0.36	0.31	0.30	0.01	0.30	0.32	0.31	0.32	0.34	0.30	0.32	0.36	0.32	0.01	18.0	0.1605
11-May-92	+26	0.28	0.29	0.36	0.34	0.28	0.29	0.36	0.32	0.31	0.01	0.31	0.32	0.33	0.35	0.34	0.29	0.33	0.34	0.33	0.01	-	-
18-May-92	+27	0.27	0.25	0.36	0.31	0.27	0.29	0.33	0.30	0.30	0.01	0.31	0.33	0.32	0.33	0.33	0.31	0.31	0.33	0.32	0.00	-	-
25-May-92	+28	0.27	0.25	0.37	0.32	0.29	0.30	0.36	0.31	0.31	0.01	0.33	0.32	0.33	0.33	0.35	0.33	0.32	0.34	0.33	0.00	-	-
01-Jun-92	+29	0.25	0.24	0.31	0.31	0.27	0.30	0.32	0.27	0.28	0.01	0.31	0.30	0.32	0.29	0.32	0.28	0.33	0.33	0.31	0.01	-	-
08-Jun-92	+30	0.24	0.24	0.33	0.31	0.25	0.30	0.35	0.28	0.29	0.01	0.32	0.33	0.31	0.31	0.31	0.31	0.34	0.35	0.32	0.01	15.0	0.0830
15-Jun-92	+31	0.25	0.25	0.34	0.33	0.27	0.30	0.36	0.32	0.30	0.01	0.34	0.36	0.31	0.33	0.33	0.33	0.36	0.37	0.34	0.01	-	-
22-Jun-92	+32	0.24	0.24	0.32	0.31	0.28	0.30	0.35	0.33	0.30	0.01	0.31	0.31	0.32	0.32	0.32	0.29	0.32	0.38	0.32	0.01	-	-
29-Jun-92	+33	0.28	0.28	0.35	0.32	0.30	0.29	0.35	0.35	0.31	0.01	0.34	0.35	0.33	0.34	0.32	0.30	0.32	0.35	0.33	0.01	-	-
06-Jul-92	+34	0.26	0.27	0.33	0.35	0.29	0.29	0.36	0.33	0.31	0.01	0.33	0.34	0.31	0.32	0.33	0.30	0.32	0.37	0.33	0.01	-	-
13-Jul-92	+35	0.27	0.26	0.32	0.34	0.28	0.30	0.35	0.35	0.31	0.01	0.36	0.33	0.30	0.30	0.35	0.32	0.32	0.35	0.33	0.01	21.0	0.2786

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done.

Appendix Table 8.5.8: Haemoglobin Concentrations (g/l) of the Buffaloes
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	119	129	135	131	112	121	127	121	124.3	2.5	125	119	123	127	111	123	119	127	121.8	1.8	24.0	0.4418
14-Oct-91	-4	127	121	135	135	111	124	134	132	127.4	2.7	121	121	127	129	111	124	117	127	122.1	2.0	-	-
21-Oct-91	-3	116	131	135	139	113	116	119	137	125.8	3.6	125	115	121	128	112	121	113	135	121.2	2.6	-	-
28-Oct-91	-2	119	119	119	127	111	119	111	139	120.6	3.0	131	111	127	119	111	127	127	135	123.6	2.9	-	-
04-Nov-91	-1	119	108	104	108	113	127	116	127	115.3	2.8	127	124	128	128	111	128	120	126	123.8	1.9	-	-
11-Nov-91	0	100	114	114	111	109	111	107	114	110.2	1.6	111	107	107	126	113	112	100	122	112.2	2.8	30.5	0.8785
18-Nov-91	+1	116	121	128	108	116	108	124	124	118.1	2.4	116	111	124	129	116	100	108	108	114.1	3.0	-	-
25-Nov-91	+2	116	108	124	100	116	100	131	131	115.9	4.1	108	124	124	108	139	108	124	108	117.9	3.8	-	-
02-Dec-91	+3	100	116	124	108	116	116	108	124	114.0	2.6	116	116	131	108	116	116	131	131	120.8	3.0	-	-
09-Dec-91	+4	108	124	131	116	116	131	129	100	119.5	3.8	108	108	116	131	124	116	147	139	120.8	6.4	-	-
16-Dec-91	+5	105	109	120	105	110	109	128	120	108.6	4.8	109	109	135	128	128	113	113	128	116.2	5.5	21.5	0.2786
23-Dec-91	+6	129	131	129	120	131	116	137	116	126.2	2.6	131	131	139	108	124	124	131	108	124.6	3.7	-	-
30-Dec-91	+7	116	131	131	124	131	124	139	124	127.5	2.4	131	139	124	124	116	131	124	131	127.5	2.4	-	-
06-Jan-92	+8	116	124	139	124	108	100	124	116	118.8	3.8	131	131	131	135	116	139	139	139	132.9	2.6	-	-
13-Jan-92	+9	131	124	124	131	116	108	131	116	122.7	2.9	131	139	124	131	117	116	139	147	130.6	3.7	-	-
20-Jan-92	+10	124	139	124	116	139	116	139	124	127.5	3.3	124	136	131	138	118	128	124	131	128.8	2.2	30.0	0.8785
27-Jan-92	+11	147	124	155	124	131	116	131	124	131.4	4.3	124	139	139	131	124	124	116	131	128.5	2.7	-	-
03-Feb-92	+12	139	100	116	131	124	100	147	124	122.7	5.5	121	137	137	129	129	121	125	139	129.9	2.3	-	-
10-Feb-92	+13	118	104	118	131	118	102	139	106	117.0	4.3	114	126	123	123	123	139	131	143	127.6	3.1	-	-
17-Feb-92	+14	108	123	123	116	113	108	133	108	116.4	3.0	105	128	143	123	120	135	121	133	126.1	3.8	-	-
24-Feb-92	+15	120	128	126	124	110	118	131	116	121.8	2.3	121	131	124	139	111	124	131	137	127.4	3.0	19.0	0.1949
02-Mar-92	+16	126	109	133	148	114	110	141	113	124.2	4.9	110	138	133	133	138	121	124	148	130.7	3.9	-	-
09-Mar-92	+17	113	102	124	135	132	109	143	113	121.4	4.8	113	123	127	128	139	117	143	139	128.6	3.7	-	-
16-Mar-92	+18	110	113	113	121	110	102	132	113	114.4	3.0	125	138	129	121	136	117	129	125	127.4	2.3	-	-
23-Mar-92	+19	90	88	113	125	101	108	121	118	108.2	4.6	116	121	121	121	131	113	124	129	122.0	1.9	-	-
30-Mar-92	+20	85	98	126	128	119	100	131	110	112.2	5.5	108	135	121	111	122	118	116	131	120.3	3.0	23.5	0.3823
06-Apr-92	+21	87	83	120	113	98	100	151	128	109.9	7.5	120	113	119	114	122	119	128	124	119.9	1.7	-	-
13-Apr-92	+22	95	98	135	113	90	118	128	105	110.4	5.3	113	113	120	113	128	113	135	128	120.4	3.0	-	-
20-Apr-92	+23	98	90	128	113	98	128	151	101	113.4	6.8	119	128	113	105	128	113	120	120	118.3	2.6	-	-
27-Apr-92	+24	98	83	128	115	90	119	135	102	108.9	6.1	105	128	113	113	128	113	120	128	118.5	2.9	-	-
04-May-92	+25	93	100	131	120	116	116	131	112	115.1	4.5	100	124	108	128	124	108	124	124	117.4	3.3	26.0	0.5737
11-May-92	+26	95	103	143	119	119	119	143	127	121.2	5.6	119	127	127	133	135	111	127	135	126.9	2.7	-	-
18-May-92	+27	100	103	138	113	106	125	143	117	118.1	5.4	123	131	120	123	126	120	110	127	122.8	2.0	-	-
25-May-92	+28	88	81	137	110	94	100	145	112	108.4	7.5	119	128	120	121	121	130	115	116	121.1	1.8	-	-
01-Jun-92	+29	93	84	122	119	91	117	147	117	111.2	6.9	128	136	136	136	119	125	127	115	127.8	2.6	-	-
08-Jun-92	+30	94	94	127	119	90	122	146	127	114.8	6.7	129	128	125	114	126	128	134	114	124.5	2.3	20.0	0.2345
15-Jun-92	+31	91	84	114	119	95	113	141	111	108.5	6.0	135	131	125	135	116	122	131	123	127.4	2.3	-	-
22-Jun-92	+32	91	84	111	118	98	115	144	106	108.3	6.2	127	126	123	137	135	128	127	126	128.5	1.6	-	-
29-Jun-92	+33	91	96	122	117	100	130	140	111	113.3	5.7	132	138	118	137	137	125	123	130	130.0	2.4	-	-
06-Jul-92	+34	92	95	125	119	108	117	132	103	111.3	4.7	132	137	113	135	125	133	129	125	128.6	2.6	-	-
13-Jul-92	+35	90	96	141	131	97	116	141	120	116.4	6.7	130	133	130	131	126	130	129	130	129.8	0.7	18.5	0.1605

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done.

Appendix Table 8.5.9: Mean Cell Volumes (fl) of the Buffaloes
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	53.4	55.6	57.5	55.1	59.0	50.0	56.3	40.5	53.4	1.94	50.1	48.3	57.1	53.0	52.1	60.6	48.9	56.8	53.4	1.46	28.0	0.7209
14-Oct-91	-4	54.6	55.9	57.1	50.7	57.1	48.0	50.3	44.7	52.3	1.52	47.1	48.7	53.7	52.8	53.8	56.3	53.7	52.1	52.3	0.99	-	-
21-Oct-91	-3	49.2	52.2	53.7	47.7	47.8	42.2	49.4	46.9	48.6	1.16	45.4	45.0	52.9	50.8	44.3	59.0	44.6	50.0	49.0	1.73	-	-
28-Oct-91	-2	51.5	50.8	50.6	48.3	73.2	44.3	53.6	46.2	52.3	2.96	46.7	50.7	47.9	53.4	49.3	59.0	45.3	46.1	49.8	1.52	-	-
04-Nov-91	-1	49.4	44.5	48.9	47.8	68.0	43.8	50.7	48.4	50.2	2.51	48.2	48.7	49.3	48.6	49.5	49.6	44.3	43.9	47.8	0.76	-	-
11-Nov-91	0	55.9	49.5	52.2	51.8	67.1	44.9	55.0	48.1	53.1	2.22	43.4	44.1	48.5	51.0	52.8	67.3	52.7	46.3	50.8	2.52	24.0	0.4418
18-Nov-91	+1	54.3	45.9	51.3	47.4	57.5	47.0	52.3	48.5	50.5	1.33	49.0	47.1	48.6	46.5	46.8	57.1	45.0	45.5	48.2	1.28	-	-
25-Nov-91	+2	60.2	51.9	54.2	55.8	57.9	48.4	56.1	52.2	54.6	1.24	49.6	48.2	50.4	49.6	50.7	58.1	49.5	42.1	49.8	1.43	-	-
02-Dec-91	+3	58.2	52.0	57.6	57.1	56.3	46.7	53.7	51.3	54.1	1.31	50.6	48.6	48.7	50.3	49.0	59.6	50.9	43.9	50.2	1.45	-	-
09-Dec-91	+4	54.8	47.9	53.3	57.0	53.8	47.3	52.6	52.5	52.4	1.09	50.9	43.6	51.2	54.2	50.5	62.1	48.8	46.7	51.0	1.81	-	-
16-Dec-91	+5	48.2	50.0	50.0	54.8	58.7	45.5	50.7	59.7	52.2	1.67	47.7	46.3	49.3	52.5	50.7	57.1	51.4	46.3	50.1	1.21	25.5	0.5054
23-Dec-91	+6	52.9	49.9	51.8	50.0	56.0	45.5	50.0	49.2	50.7	1.02	52.9	48.4	49.6	51.3	48.7	60.5	51.2	59.3	52.8	1.55	-	-
30-Dec-91	+7	55.9	49.7	52.0	52.6	50.4	46.6	51.9	52.2	51.4	0.88	49.2	49.0	54.0	53.4	46.9	50.7	48.4	45.6	49.6	0.98	-	-
06-Jan-92	+8	49.4	46.3	50.5	52.6	51.2	44.5	39.0	49.5	47.9	1.47	48.4	49.1	49.4	50.6	48.9	57.9	42.0	42.9	48.7	1.61	-	-
13-Jan-92	+9	52.5	46.8	51.5	50.8	53.0	43.5	49.5	67.6	51.9	2.35	51.0	46.3	51.1	51.5	46.6	56.3	45.3	46.3	49.3	1.26	-	-
20-Jan-92	+10	54.9	46.9	51.0	52.9	55.1	46.8	58.5	52.9	52.4	1.34	50.0	48.9	49.7	55.7	50.1	58.7	48.6	47.8	51.2	1.28	27.0	0.6454
27-Jan-92	+11	48.8	50.0	55.4	52.4	51.6	48.1	59.2	49.9	51.9	1.24	48.5	45.7	53.1	51.4	51.0	58.7	49.3	51.0	51.1	1.26	-	-
03-Feb-92	+12	51.0	51.1	54.1	51.5	51.0	49.2	51.3	52.8	51.5	0.48	52.2	44.0	50.8	51.4	47.6	53.4	49.8	47.2	49.6	1.02	-	-
10-Feb-92	+13	45.8	58.5	52.4	49.4	49.8	47.6	51.5	48.9	50.5	1.27	50.4	46.5	51.1	52.9	48.5	55.5	49.4	45.6	50.0	1.08	-	-
17-Feb-92	+14	53.8	48.9	54.7	50.0	46.4	48.7	52.7	50.7	50.7	0.94	48.1	45.8	50.3	50.6	48.3	56.3	48.3	46.8	49.3	1.07	-	-
24-Feb-92	+15	50.6	50.9	50.1	52.4	48.6	49.6	49.4	50.4	50.2	0.38	46.9	49.3	53.0	52.8	50.0	54.3	47.0	46.5	50.0	1.02	28.0	0.7209
02-Mar-92	+16	52.4	52.4	53.7	50.0	50.2	48.5	53.3	52.0	51.6	0.60	50.0	47.0	49.6	55.1	48.7	56.9	50.7	47.1	50.6	1.19	-	-
09-Mar-92	+17	50.4	51.7	49.7	51.5	45.4	48.9	49.8	51.6	49.9	0.69	52.4	46.1	55.2	48.4	53.1	67.7	49.2	46.9	52.4	2.30	-	-
16-Mar-92	+18	54.2	55.3	51.3	47.6	44.3	47.1	47.6	48.7	49.5	1.25	48.2	41.3	50.1	50.8	49.3	54.1	46.4	45.8	48.3	1.27	-	-
23-Mar-92	+19	57.7	57.7	57.2	50.2	51.8	54.0	54.1	49.3	54.0	1.11	47.1	41.8	50.0	56.7	47.1	54.1	49.3	48.7	49.3	1.50	-	-
30-Mar-92	+20	61.4	59.1	52.3	48.5	47.1	50.4	48.0	57.7	53.1	1.84	48.4	50.8	51.1	53.7	52.9	59.6	51.6	46.5	51.8	1.30	31.0	0.9591
06-Apr-92	+21	60.0	59.1	56.0	50.4	50.6	47.0	48.7	50.4	52.8	1.63	46.5	53.8	46.4	52.4	50.1	64.2	46.8	47.3	50.9	2.01	-	-
13-Apr-92	+22	64.9	58.8	55.9	55.1	52.7	49.5	57.1	56.3	56.3	1.48	45.6	51.6	54.1	49.2	48.3	58.6	49.2	50.1	50.9	1.32	-	-
20-Apr-92	+23	60.6	66.1	54.1	54.1	53.6	47.6	47.7	52.6	54.6	2.06	49.2	53.2	50.0	50.6	50.8	56.1	54.4	53.2	52.2	0.80	-	-
27-Apr-92	+24	57.7	57.5	51.2	51.7	51.2	46.7	49.7	52.4	52.3	1.23	54.9	47.4	46.2	54.4	49.6	55.8	48.9	46.4	50.4	1.32	-	-
04-May-92	+25	57.9	53.6	57.4	59.0	52.8	52.1	51.3	53.4	54.7	0.97	53.1	49.2	53.9	49.4	48.2	58.3	47.4	48.8	51.0	1.23	15.0	0.0830
11-May-92	+26	53.0	53.2	53.3	55.7	53.3	49.0	48.6	49.6	52.0	0.85	53.0	53.2	56.3	47.4	50.0	48.9	48.3	45.3	50.3	1.19	-	-
18-May-92	+27	55.5	56.8	53.5	50.8	54.7	48.1	49.6	50.7	52.5	1.03	54.4	50.8	55.3	52.8	49.6	53.0	50.7	48.5	51.9	0.78	-	-
25-May-92	+28	57.0	58.1	54.1	49.1	56.9	46.2	48.0	51.0	52.6	1.52	55.2	49.2	48.4	49.5	50.0	51.3	46.9	48.1	49.8	0.83	-	-
01-Jun-92	+29	54.9	52.4	48.6	50.8	49.1	45.5	48.1	47.6	49.6	0.99	48.7	47.2	48.5	49.4	48.7	48.7	46.3	49.0	48.3	0.34	-	-
08-Jun-92	+30	52.2	53.5	54.1	47.7	49.5	45.3	46.5	46.9	49.5	1.12	48.1	48.8	49.2	53.1	46.3	47.7	45.1	50.2	48.6	0.81	27.5	0.6454
15-Jun-92	+31	58.1	54.2	55.7	50.4	48.8	42.9	50.7	46.8	50.9	1.64	55.3	55.0	47.8	53.8	48.1	55.2	45.9	50.0	51.4	1.28	-	-
22-Jun-92	+32	53.3	48.3	49.2	44.8	49.7	48.1	47.3	47.1	48.5	0.81	49.2	47.0	51.9	48.7	51.1	49.2	46.7	49.7	49.2	0.59	-	-
29-Jun-92	+33	51.4	51.9	54.7	50.8	51.7	49.2	46.9	53.4	51.2	0.80	53.4	55.6	44.9	53.7	52.6	51.7	43.0	52.9	51.0	1.49	-	-
06-Jul-92	+34	52.5	52.9	51.6	57.4	52.3	42.5	50.1	50.0	51.2	1.38	51.6	48.8	48.8	50.5	53.2	58.8	47.3	47.2	50.8	1.27	-	-
13-Jul-92	+35	47.5	55.5	49.1	53.9	50.8	45.0	48.7	51.9	50.3	1.14	50.9	46.6	47.4	45.9	47.0	58.2	45.2	45.6	48.3	1.44	19.0	0.1949

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done.

Appendix Table 8.5.10: Mean Cell Haemoglobin (pg) of the Buffaloes
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	17.6	21.7	21.0	20.6	20.7	20.8	19.9	16.3	19.8	0.6	20.2	19.1	24.3	19.8	17.1	23.2	17.6	19.5	20.1	0.8	27.5	0.6554
14-Oct-91	-4	19.8	19.4	21.4	19.0	19.9	19.8	19.3	17.4	19.5	0.4	17.9	16.8	31.0	20.0	18.1	20.5	19.0	16.9	20.0	1.5	-	-
21-Oct-91	-3	16.8	19.6	20.1	19.0	17.4	17.5	16.8	18.4	18.2	0.4	16.7	15.7	23.6	19.6	15.5	21.1	15.3	16.8	18.0	1.0	-	-
28-Oct-91	-2	18.0	17.3	17.2	17.1	26.2	18.2	16.6	19.4	18.8	1.0	19.1	17.6	18.5	18.7	18.3	24.2	16.9	16.4	18.7	0.8	-	-
04-Nov-91	-1	18.4	16.1	15.0	15.7	25.6	16.8	17.3	17.6	17.8	1.1	18.5	17.7	18.0	18.8	18.4	20.4	17.1	14.6	17.9	0.6	-	-
11-Nov-91	0	16.0	16.7	18.1	16.9	23.0	15.5	18.4	16.7	17.7	0.8	16.0	15.8	14.9	19.5	19.2	21.5	16.0	14.4	17.2	0.8	25.0	0.5054
18-Nov-91	+1	17.0	17.4	19.2	16.6	22.2	15.4	18.0	18.2	18.0	0.7	17.7	15.9	17.7	18.2	17.0	19.1	15.2	14.5	16.9	0.5	-	-
25-Nov-91	+2	19.9	17.5	20.3	17.0	21.7	15.2	19.9	20.8	19.0	0.7	17.9	17.5	18.3	17.3	20.2	20.9	18.0	13.0	17.9	0.8	-	-
02-Dec-91	+3	18.3	18.9	21.6	18.2	20.4	17.5	16.2	18.6	18.7	0.6	20.2	18.8	20.6	18.1	15.8	21.6	19.7	15.2	18.8	0.8	-	-
09-Dec-91	+4	18.0	19.7	22.6	19.4	20.1	18.8	17.4	17.0	19.1	0.6	19.0	16.9	18.6	23.0	17.8	21.8	20.5	17.1	19.3	0.7	-	-
16-Dec-91	+5	16.4	16.5	19.4	17.0	17.9	16.5	19.1	20.5	17.9	0.5	16.8	16.3	20.2	21.0	18.5	20.2	15.7	16.0	18.1	0.7	30.0	0.8785
23-Dec-91	+6	18.0	18.7	19.7	16.6	21.7	17.6	16.7	18.4	18.4	0.5	21.1	19.3	21.6	17.4	17.7	21.4	17.7	17.8	19.2	0.6	-	-
30-Dec-91	+7	17.1	18.1	19.5	18.1	18.9	18.0	17.6	19.6	18.4	0.3	20.2	19.5	18.1	18.9	16.0	18.5	16.2	16.2	17.9	0.5	-	-
06-Jan-92	+8	16.4	17.3	20.7	18.6	15.8	15.4	16.1	17.4	17.2	0.6	20.5	19.6	18.0	20.1	17.2	22.4	17.7	15.7	18.9	0.7	-	-
13-Jan-92	+9	18.2	17.0	18.7	18.6	17.6	15.7	18.1	20.1	18.0	0.4	19.7	18.9	17.5	19.3	16.0	18.7	18.6	18.4	18.4	0.4	-	-
20-Jan-92	+10	17.4	19.8	18.6	17.5	21.9	18.7	20.9	19.8	19.3	0.5	18.7	19.6	17.6	22.0	16.9	20.2	16.7	16.5	18.5	0.6	23.5	0.3823
27-Jan-92	+11	19.9	18.2	23.1	18.5	19.4	19.2	21.0	20.6	20.0	0.5	17.6	19.3	19.5	17.8	17.1	20.2	16.3	18.1	18.2	0.4	-	-
03-Feb-92	+12	17.7	16.0	17.4	19.3	18.0	17.0	18.4	18.1	17.8	0.3	18.1	17.2	18.3	17.4	17.1	18.5	16.9	17.8	17.7	0.2	-	-
10-Feb-92	+13	15.0	19.6	16.3	17.0	17.3	16.1	17.9	15.7	16.9	0.5	16.9	17.8	17.4	17.1	17.5	21.4	18.0	18.1	18.0	0.5	-	-
17-Feb-92	+14	15.3	18.8	17.2	15.6	15.9	16.9	16.7	15.6	16.5	0.4	14.9	17.3	20.0	16.8	16.2	20.6	15.3	16.0	17.1	0.7	-	-
24-Feb-92	+15	18.5	22.5	18.6	17.1	17.3	19.5	16.2	18.3	18.5	0.6	17.3	18.5	17.3	19.3	13.9	19.7	17.6	15.9	17.4	0.6	25.5	0.5054
02-Mar-92	+16	21.2	19.7	19.9	20.0	16.9	17.9	19.2	17.3	19.0	0.5	17.3	16.6	17.8	20.9	18.2	18.5	16.6	16.6	17.8	0.5	-	-
09-Mar-92	+17	19.0	18.8	18.7	18.9	18.7	18.4	19.2	18.2	18.7	0.1	17.9	16.2	17.5	16.7	20.5	21.9	19.5	17.2	18.4	0.7	-	-
16-Mar-92	+18	22.0	24.1	17.6	16.9	17.4	17.2	18.5	17.8	18.9	0.9	18.8	17.2	18.4	17.6	20.3	19.2	17.5	17.3	18.3	0.4	-	-
23-Mar-92	+19	19.3	18.9	18.6	17.9	19.3	21.7	18.2	20.8	19.3	0.4	17.0	15.3	17.8	20.2	19.3	18.6	17.9	17.9	18.0	0.5	-	-
30-Mar-92	+20	19.3	23.2	20.6	19.4	20.7	17.3	17.5	19.9	19.7	0.6	17.5	20.7	19.9	18.1	20.2	20.7	18.1	17.0	19.0	0.5	28.0	0.7209
06-Apr-92	+21	17.9	18.1	19.8	17.8	19.2	17.3	19.3	19.5	18.6	0.3	18.7	19.0	17.3	18.1	18.5	21.9	18.1	16.8	18.5	0.5	-	-
13-Apr-92	+22	22.1	23.0	23.0	21.4	19.8	21.6	20.3	20.5	21.5	0.4	16.6	18.2	19.7	17.4	19.9	19.5	20.8	18.9	18.9	0.5	-	-
20-Apr-92	+23	20.5	21.3	21.0	20.3	20.2	23.5	19.4	19.7	20.7	0.4	19.5	21.3	17.6	17.8	19.7	19.8	19.8	19.4	19.4	0.4	-	-
27-Apr-92	+24	20.2	19.0	19.8	19.9	18.5	20.6	18.2	18.5	19.3	0.3	18.6	19.6	17.4	19.8	19.8	19.7	17.8	18.0	18.8	0.3	-	-
04-May-92	+25	19.9	20.7	21.5	23.7	21.9	20.8	18.7	19.3	20.8	0.5	19.8	19.0	18.8	19.7	17.5	21.0	18.3	20.8	19.4	0.4	15.5	0.0830
11-May-92	+26	18.0	19.0	21.2	19.5	22.7	20.1	19.3	19.7	19.9	0.5	20.4	21.1	21.7	18.0	19.9	18.7	18.6	18.0	19.5	0.5	-	-
18-May-92	+27	20.5	23.4	20.5	18.5	21.4	20.8	21.6	19.8	20.8	0.5	21.7	20.2	20.8	19.7	19.0	20.6	18.1	18.7	19.8	0.4	-	-
25-May-92	+28	18.6	18.9	20.1	16.9	18.3	15.4	19.3	18.4	18.2	0.5	19.9	19.7	17.6	18.1	17.2	20.2	16.8	16.4	18.2	0.5	-	-
01-Jun-92	+29	20.3	18.3	19.1	19.6	16.5	17.7	22.1	20.7	19.3	0.6	20.1	21.4	20.5	23.1	18.1	21.8	17.9	17.1	20.0	0.7	-	-
08-Jun-92	+30	20.4	20.9	20.8	18.4	17.8	18.4	19.3	21.3	19.7	0.5	19.3	18.9	19.8	19.5	18.8	19.6	17.8	16.4	18.8	0.4	22.0	0.3282
15-Jun-92	+31	21.1	18.1	18.7	18.1	17.2	16.2	19.9	16.3	18.2	0.6	22.0	20.1	19.3	22.0	16.9	20.4	16.7	16.7	19.3	0.7	-	-
22-Jun-92	+32	20.2	17.0	17.0	17.1	17.4	18.4	19.5	15.1	17.7	0.5	20.1	19.1	19.9	20.9	21.5	21.7	18.6	16.4	19.8	0.6	-	-
29-Jun-92	+33	16.7	17.7	19.1	18.6	17.2	22.0	18.7	17.0	18.4	0.6	20.8	21.8	16.0	21.6	22.5	21.6	16.6	19.6	20.1	0.8	-	-
06-Jul-92	+34	18.6	18.6	19.5	19.5	19.4	17.1	18.4	15.6	18.3	0.4	20.7	19.6	17.8	21.4	20.1	26.1	19.1	15.9	20.1	1.0	-	-
13-Jul-92	+35	15.8	20.5	21.6	20.7	17.6	17.4	19.6	17.8	18.9	0.7	18.4	18.8	20.5	20.1	16.8	23.6	18.2	16.9	19.2	0.7	31.5	0.9591

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done.

Appendix Table 8.5.11: Mean Cell Haemoglobin Concentrations (g/l) of the Buffaloes
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	330	391	366	373	351	416	353	402	372.8	9.5	404	396	426	374	328	383	360	344	376.8	10.7	29.0	0.7984
14-Oct-91	-4	363	347	374	374	348	412	384	389	373.8	7.2	379	345	479	380	336	364	354	325	370.2	15.8	-	-
21-Oct-91	-3	341	375	374	397	365	414	340	391	374.8	8.6	369	349	446	386	350	357	342	337	367.1	11.8	-	-
28-Oct-91	-2	350	340	340	353	359	411	309	421	360.5	12.4	410	347	385	350	371	410	374	355	375.3	8.2	-	-
04-Nov-91	-1	372	361	307	328	377	385	341	363	354.1	8.8	385	364	364	386	371	411	386	331	374.9	7.7	-	-
11-Nov-91	0	286	336	347	326	342	346	335	347	333.1	6.7	369	358	306	383	363	319	303	312	339.3	10.6	28.0	0.7209
18-Nov-91	+1	313	379	375	349	386	328	344	375	356.2	8.8	362	338	364	391	362	335	338	318	351.1	7.6	-	-
25-Nov-91	+2	331	338	375	305	374	314	355	398	348.8	10.7	361	364	364	349	398	361	364	309	358.6	8.0	-	-
02-Dec-91	+3	314	362	375	318	362	374	301	364	346.3	9.9	400	386	424	361	322	362	386	346	373.5	10.7	-	-
09-Dec-91	+4	328	412	424	341	374	398	332	324	366.6	13.5	373	386	362	424	353	351	420	366	379.5	9.4	-	-
16-Dec-91	+5	340	330	388	310	305	363	376	344	344.5	9.9	352	353	411	400	366	353	305	346	360.5	10.9	21.5	0.2786
23-Dec-91	+6	340	375	380	332	386	386	334	374	363.5	7.9	398	398	435	338	364	353	346	301	366.6	13.9	-	-
30-Dec-91	+7	305	365	375	344	375	386	339	375	358.1	8.9	411	398	334	353	341	365	334	355	361.4	9.5	-	-
06-Jan-92	+8	331	375	409	353	309	347	412	351	361.0	11.9	424	398	365	398	351	386	422	366	388.8	8.8	-	-
13-Jan-92	+9	346	364	364	365	331	361	365	297	349.1	8.0	386	409	344	375	344	331	409	397	374.5	10.3	-	-
20-Jan-92	+10	317	422	364	331	398	400	357	375	370.3	11.8	375	400	355	395	338	345	344	346	362.0	8.1	26.0	0.5737
27-Jan-92	+11	408	364	418	353	375	400	355	412	385.7	8.8	364	422	366	346	334	344	331	355	357.7	9.5	-	-
03-Feb-92	+12	348	314	322	375	353	347	358	344	345.1	6.5	347	391	360	340	359	347	339	376	357.3	6.1	-	-
10-Feb-92	+13	328	335	312	344	347	339	347	322	334.3	4.2	336	383	340	323	361	386	363	397	361.1	8.7	-	-
17-Feb-92	+14	284	384	315	312	342	348	317	308	326.4	10.2	310	376	397	332	335	366	317	341	346.8	10.0	-	-
24-Feb-92	+15	365	441	370	325	356	394	329	362	368.0	12.3	368	375	325	366	279	364	375	342	349.4	11.1	28.5	0.7209
02-Mar-92	+16	405	376	369	400	336	368	360	331	368.3	8.7	345	354	359	380	373	326	327	353	352.2	6.4	-	-
09-Mar-92	+17	376	363	376	366	412	376	386	353	376.1	5.8	342	351	317	346	387	324	397	366	353.9	9.3	-	-
16-Mar-92	+18	407	435	344	356	392	365	389	366	381.8	9.9	390	417	367	346	413	355	378	378	380.6	8.3	-	-
23-Mar-92	+19	335	327	324	357	373	401	336	423	359.6	12.1	362	367	356	356	410	344	363	367	365.7	6.4	-	-
30-Mar-92	+20	315	392	394	401	440	344	365	344	374.3	13.2	361	408	389	338	382	348	351	365	367.5	7.8	28.5	0.7209
06-Apr-92	+21	298	307	354	353	378	369	396	388	355.4	11.9	401	353	372	345	370	340	388	355	365.5	7.0	-	-
13-Apr-92	+22	341	391	411	389	376	436	355	363	382.9	10.2	364	353	365	353	413	332	423	376	372.4	10.3	-	-
20-Apr-92	+23	337	323	388	376	376	494	407	375	384.5	17.1	396	400	353	351	388	353	365	365	371.3	6.7	-	-
27-Apr-92	+24	349	331	388	385	361	441	366	353	371.9	11.1	340	413	376	364	400	353	365	388	374.8	8.1	-	-
04-May-92	+25	344	386	375	402	414	400	365	362	380.9	7.9	335	386	349	399	364	361	386	344	365.4	7.6	18.5	0.1605
11-May-92	+26	340	356	397	350	426	411	397	397	384.4	10.3	384	397	385	380	397	384	385	397	388.7	2.4	-	-
18-May-92	+27	370	412	383	365	391	432	435	390	397.1	8.8	398	398	376	374	383	388	356	385	382.2	4.5	-	-
25-May-92	+28	326	324	371	344	322	334	403	360	348.2	9.3	360	400	364	366	345	394	358	342	366.0	6.9	-	-
01-Jun-92	+29	370	350	394	385	336	389	460	434	389.6	13.5	413	453	424	468	371	448	386	350	414.1	13.9	-	-
08-Jun-92	+30	391	390	385	385	360	406	416	455	398.5	9.3	402	387	403	366	405	411	394	326	386.8	9.3	30.0	0.8785
15-Jun-92	+31	363	335	335	359	353	377	392	348	357.8	6.6	398	365	403	408	352	370	364	334	374.3	8.8	-	-
22-Jun-92	+32	379	351	346	381	349	382	412	321	365.1	9.5	408	406	383	429	421	442	398	331	402.3	11.3	-	-
29-Jun-92	+33	326	342	349	365	333	448	399	318	360.0	14.5	389	393	357	403	428	418	386	371	393.0	7.6	-	-
06-Jul-92	+34	355	351	377	340	371	403	367	312	359.6	8.9	401	402	365	423	377	443	403	336	393.9	11.1	-	-
13-Jul-92	+35	333	370	440	384	346	387	402	343	375.5	11.7	361	403	433	438	359	406	403	371	396.8	10.2	19.0	0.1949

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done.

Appendix Table 8.5.12: Total WBC Counts (x109 cells/litre of blood) of Buffaloes Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	9.4	8.7	6.2	5.1	10.3	8.3	11.5	12.2	8.96	0.81	8.2	10.4	5.2	12.6	7.8	9.5	14.9	11.0	9.93	1.00	26.0	0.5737
14-Oct-91	-4	8.5	8.7	6.3	5.2	9.2	9.7	10.3	11.1	8.61	0.66	9.1	8.1	8.9	12.9	8.5	7.2	11.1	7.4	9.15	0.64	-	-
21-Oct-91	-3	8.8	11.5	9.6	5.8	7.3	10.3	13.0	12.6	9.86	0.83	10.9	14.8	8.9	15.1	5.6	10.0	11.6	12.0	11.12	1.03	-	-
28-Oct-91	-2	7.1	10.5	6.8	4.0	9.6	9.8	10.1	10.6	8.55	0.78	5.9	11.9	9.3	13.9	14.8	8.2	10.7	10.7	10.65	0.96	-	-
04-Nov-91	-1	7.4	10.4	6.6	5.3	6.2	11.1	10.7	9.8	8.44	0.76	8.2	11.6	9.2	12.5	10.9	7.9	9.9	11.8	10.25	0.57	-	-
11-Nov-91	0	7.6	10.1	5.6	5.2	8.5	9.9	9.8	9.0	8.22	0.64	9.6	12.2	8.2	13.1	7.9	7.4	7.8	9.4	9.45	0.70	27.0	0.6454
18-Nov-91	+1	7.5	11.4	7.7	9.9	10.1	9.1	7.8	10.0	9.19	0.47	8.2	8.2	8.4	8.1	8.9	7.9	8.5	10.9	8.64	0.31	-	-
25-Nov-91	+2	12.6	9.5	9.0	10.4	8.0	11.8	10.9	12.2	10.56	0.54	8.7	10.5	7.8	5.4	7.6	8.5	10.1	12.5	8.90	0.71	-	-
02-Dec-91	+3	9.3	10.2	10.3	9.6	10.0	9.3	11.3	12.2	10.29	0.34	9.2	9.6	9.3	8.5	10.5	9.0	11.4	10.3	9.73	0.31	-	-
09-Dec-91	+4	9.8	12.9	9.7	10.0	11.4	8.4	11.7	11.2	10.64	0.48	8.8	9.9	7.8	8.3	8.1	8.1	12.5	10.8	9.29	0.55	-	-
16-Dec-91	+5	10.9	11.0	11.4	9.6	10.8	8.6	11.7	11.7	10.72	0.36	7.7	10.7	9.6	8.7	10.6	8.5	12.8	10.3	9.87	0.53	16.5	0.1049
23-Dec-91	+6	8.0	11.1	9.2	10.7	9.3	9.7	11.8	11.9	10.21	0.45	6.8	11.4	12.1	6.4	9.5	10.5	14.7	11.9	10.41	0.93	-	-
30-Dec-91	+7	9.7	10.1	9.3	10.3	10.4	9.6	11.2	11.5	10.25	0.26	8.7	11.4	11.9	5.8	10.2	9.9	12.0	9.9	9.98	0.67	-	-
06-Jan-92	+8	10.8	11.6	9.8	9.1	11.2	8.8	10.8	9.3	10.19	0.34	7.7	11.5	7.6	5.7	11.2	9.6	10.9	10.8	9.38	0.70	-	-
13-Jan-92	+9	8.7	11.2	9.3	10.6	9.7	7.9	10.5	10.3	9.77	0.36	7.1	10.2	6.9	5.7	10.0	8.5	11.1	8.0	8.45	0.61	-	-
20-Jan-92	+10	9.9	10.3	11.5	8.4	10.5	9.0	10.2	9.7	9.93	0.31	8.1	10.2	7.5	8.5	10.1	9.8	9.8	9.3	9.16	0.33	17.5	0.1304
27-Jan-92	+11	8.3	10.3	10.0	9.8	9.9	9.4	9.2	8.0	9.37	0.27	7.8	9.7	8.3	7.7	9.9	9.8	7.6	8.1	8.62	0.33	-	-
03-Feb-92	+12	9.4	11.7	9.3	10.9	8.8	7.8	11.0	9.6	9.80	0.43	7.7	9.1	9.5	6.4	11.1	9.9	12.3	8.7	9.34	0.62	-	-
10-Feb-92	+13	8.5	9.3	9.7	10.0	9.6	8.0	9.4	7.6	9.00	0.29	8.1	9.5	6.9	7.1	9.8	9.1	11.1	8.5	8.75	0.47	-	-
17-Feb-92	+14	9.5	10.0	10.4	11.5	11.5	8.2	9.9	9.2	10.02	0.37	7.1	9.5	8.7	7.6	10.8	9.6	11.4	9.5	9.28	0.48	-	-
24-Feb-92	+15	8.6	9.3	10.6	9.2	10.4	8.5	9.9	9.4	9.49	0.26	8.3	8.8	7.2	7.1	8.6	8.0	10.4	9.4	8.47	0.36	14.5	0.0650
02-Mar-92	+16	13.2	9.5	9.5	10.1	9.0	7.7	9.0	8.2	9.53	0.56	9.3	8.8	7.6	6.0	9.4	8.6	8.8	9.3	8.49	0.38	-	-
09-Mar-92	+17	7.9	8.9	9.4	10.6	11.5	5.8	9.5	9.2	9.10	0.56	5.4	8.1	6.2	7.7	11.4	9.3	10.3	8.5	8.37	0.66	-	-
16-Mar-92	+18	12.1	9.4	8.4	10.4	10.5	6.8	9.5	9.1	9.53	0.52	10.9	7.6	5.6	8.8	9.9	8.4	8.8	8.9	8.62	0.52	-	-
23-Mar-92	+19	8.9	7.7	8.6	9.9	8.4	5.8	9.0	8.9	8.40	0.40	7.0	7.2	8.5	7.9	10.8	8.0	8.3	8.1	8.23	0.38	-	-
30-Mar-92	+20	7.8	7.6	8.3	11.0	10.4	6.4	8.8	9.3	8.71	0.51	5.9	8.0	6.7	8.5	9.8	9.5	9.2	7.5	8.15	0.46	26.0	0.5737
06-Apr-92	+21	8.2	8.2	7.8	10.4	7.9	6.1	10.3	9.9	8.60	0.50	8.0	7.5	6.4	6.7	9.7	9.3	9.1	9.1	8.23	0.42	-	-
13-Apr-92	+22	9.1	8.9	9.5	10.9	8.9	7.2	10.4	9.9	9.35	0.37	6.3	7.0	8.0	7.0	9.4	9.0	9.4	7.5	7.96	0.39	-	-
20-Apr-92	+23	8.7	9.2	10.0	11.2	7.7	7.6	9.3	10.5	9.28	0.42	6.5	7.2	7.7	6.6	8.5	7.9	9.2	7.0	7.58	0.31	-	-
27-Apr-92	+24	7.6	8.6	9.4	10.7	7.7	6.9	9.5	9.9	8.78	0.43	5.7	7.9	6.7	7.1	9.4	7.8	10.4	7.7	7.83	0.49	-	-
04-May-92	+25	8.2	8.6	8.7	11.5	9.3	7.4	9.3	9.1	9.00	0.40	6.5	9.0	7.1	7.2	9.1	8.5	9.6	8.3	8.15	0.36	19.5	0.1949
11-May-92	+26	8.6	8.1	9.3	12.2	8.6	7.0	9.5	10.1	9.18	0.51	7.3	7.5	6.6	6.5	9.5	8.6	10.7	8.5	8.15	0.48	-	-
18-May-92	+27	8.6	9.1	9.3	12.0	11.8	8.8	8.3	10.3	9.77	0.47	6.9	7.4	8.5	8.9	10.1	8.9	10.9	8.1	8.72	0.44	-	-
25-May-92	+28	8.3	9.2	9.6	10.6	9.4	8.4	10.3	9.3	9.40	0.27	8.0	6.6	7.2	7.1	9.6	9.0	9.6	8.7	8.23	0.39	-	-
01-Jun-92	+29	8.8	10.2	9.6	11.8	10.8	6.6	10.0	9.2	9.63	0.51	7.1	7.3	7.4	6.8	9.7	8.8	10.3	8.3	8.22	0.42	-	-
08-Jun-92	+30	8.7	9.2	9.2	11.7	9.1	7.7	9.6	9.8	9.37	0.37	6.8	6.7	9.0	8.0	10.1	8.4	10.7	6.6	8.28	0.52	18.0	0.1605
15-Jun-92	+31	9.5	8.6	10.1	12.6	8.3	7.8	9.5	10.1	9.56	0.49	7.9	7.0	7.0	8.2	9.8	8.6	9.8	9.1	8.42	0.37	-	-
22-Jun-92	+32	9.3	8.5	10.8	12.9	8.9	7.4	10.6	10.5	9.86	0.57	6.3	7.8	9.3	8.4	9.4	8.1	9.9	9.6	8.58	0.39	-	-
29-Jun-92	+33	9.6	9.5	13.1	16.2	8.2	9.1	8.5	11.3	10.69	0.91	6.2	6.7	9.4	7.0	12.9	10.0	11.5	10.9	9.34	0.82	-	-
06-Jul-92	+34	9.3	7.4	10.5	11.4	8.8	7.8	9.9	8.8	9.23	0.44	8.3	6.0	7.1	5.4	10.7	8.2	9.9	9.8	8.18	0.63	-	-
13-Jul-92	+35	10.9	9.2	11.1	10.3	8.3	8.5	9.5	11.4	9.91	0.40	8.5	8.3	7.3	6.6	10.0	7.4	10.8	9.8	8.60	0.49	15.0	0.0830

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done.

Appendix Table 8.5.13: Eosinophil Counts (x109 cells/litre of blood) of Buffaloes Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	0.06	0.08	0.08	0.05	0.12	0.07	0.08	0.10	0.08	0.01	0.05	0.05	0.12	0.02	0.11	0.04	0.08	0.14	0.08	0.01	28.0	0.7209
14-Oct-91	-4	0.03	0.07	0.09	0.03	0.11	0.04	0.02	0.11	0.06	0.01	0.01	0.02	0.13	0.03	0.10	0.02	0.03	0.17	0.06	0.02	-	-
21-Oct-91	-3	0.03	0.02	0.03	0.08	0.08	0.11	0.04	0.10	0.06	0.01	0.02	0.03	0.08	0.02	0.13	0.01	0.05	0.20	0.07	0.02	-	-
28-Oct-91	-2	0.01	0.01	0.01	0.01	0.14	0.12	0.03	0.15	0.06	0.02	0.03	0.05	0.06	0.02	0.12	0.07	0.12	0.10	0.07	0.01	-	-
04-Nov-91	-1	0.06	0.03	0.04	0.07	0.18	0.13	0.06	0.03	0.07	0.02	0.01	0.11	0.02	0.03	0.13	0.04	0.04	0.08	0.06	0.01	-	-
11-Nov-91	0	0.06	0.02	0.01	0.01	0.11	0.06	0.06	0.04	0.04	0.01	0.07	0.06	0.08	0.02	0.10	0.02	0.07	0.08	0.06	0.01	18.5	0.1605
18-Nov-91	+1	0.05	0.04	0.02	0.06	0.10	0.13	0.05	0.03	0.06	0.01	0.02	0.10	0.05	0.03	0.04	0.04	0.03	0.07	0.05	0.01	-	-
25-Nov-91	+2	0.10	0.02	0.01	0.01	0.12	0.40	0.03	0.13	0.10	0.04	0.02	0.05	0.03	0.06	0.05	0.10	0.08	0.08	0.06	0.01	-	-
02-Dec-91	+3	0.40	0.04	0.05	0.04	0.10	0.16	0.08	0.14	0.13	0.04	0.10	0.10	0.03	0.06	0.03	0.02	0.11	0.07	0.06	0.01	20.0	0.2345
09-Dec-91	+4	0.53	0.08	0.03	0.13	0.20	0.27	0.09	0.11	0.18	0.05	0.02	0.09	0.06	0.08	0.09	0.11	0.04	0.17	0.08	0.02	17.0	0.1304
16-Dec-91	**+5**	0.40	0.22	0.17	0.38	0.41	0.13	0.66	0.16	0.32	0.06	0.07	0.04	0.13	0.02	0.05	0.02	0.08	0.10	0.06	0.01	0.5	0.0002
23-Dec-91	**+6**	0.32	0.19	0.12	0.23	0.25	0.22	0.27	0.16	0.22	0.02	0.08	0.07	0.15	0.03	0.13	0.08	0.16	0.06	0.09	0.01	-	-
30-Dec-91	**+7**	0.21	0.15	0.05	0.78	0.17	0.27	0.14	0.10	0.23	0.08	0.06	0.03	0.15	0.06	0.07	0.09	0.12	0.17	0.09	0.02	-	-
06-Jan-92	**+8**	0.19	0.11	0.15	0.39	0.20	0.24	0.20	0.23	0.21	0.03	0.07	0.09	0.08	0.05	0.02	0.04	0.03	0.17	0.07	0.02	-	-
13-Jan-92	**+9**	0.17	0.13	0.10	0.69	0.18	0.25	0.45	0.14	0.26	0.07	0.08	0.05	0.11	0.05	0.05	0.03	0.08	0.09	0.07	0.01	-	-
20-Jan-92	**+10**	0.36	0.25	0.10	0.27	0.15	0.26	0.25	0.13	0.22	0.03	0.08	0.08	0.10	0.13	0.09	0.04	0.09	0.12	0.09	0.01	3.0	0.0011
27-Jan-92	**+11**	0.48	0.45	0.12	0.44	0.09	0.19	0.10	0.31	0.27	0.06	0.05	0.10	0.13	0.08	0.04	0.06	0.11	0.11	0.08	0.01	-	-
03-Feb-92	**+12**	0.39	0.17	0.17	0.14	0.19	0.15	0.20	0.27	0.21	0.03	0.05	0.09	0.12	0.06	0.03	0.05	0.03	0.13	0.07	0.01	-	-
10-Feb-92	**+13**	0.13	0.12	0.16	0.09	0.14	0.50	0.30	0.19	0.20	0.05	0.04	0.10	0.11	0.05	0.05	0.08	0.10	0.17	0.09	0.01	-	-
17-Feb-92	**+14**	0.36	0.24	0.20	0.07	0.09	0.27	0.13	0.08	0.18	0.03	0.03	0.05	0.10	0.11	0.06	0.03	0.12	0.19	0.09	0.02	-	-
24-Feb-92	**+15**	0.04	0.12	0.17	0.13	0.15	0.15	0.16	0.13	0.13	0.01	0.11	0.06	0.09	0.10	0.02	0.10	0.14	0.11	0.09	0.01	10.0	0.0207
02-Mar-92	**+16**	0.02	0.17	0.23	0.19	0.21	0.14	0.20	0.20	0.17	0.02	0.11	0.04	0.17	0.06	0.05	0.09	0.08	0.09	0.08	0.01	-	-
09-Mar-92	**+17**	0.10	0.31	0.08	0.15	0.38	0.39	0.18	0.13	0.22	0.04	0.06	0.08	0.11	0.06	0.09	0.04	0.12	0.10	0.08	0.01	-	-
16-Mar-92	**+18**	0.07	0.08	0.06	0.17	0.25	0.34	0.07	0.16	0.15	0.03	0.08	0.09	0.12	0.06	0.07	0.08	0.11	0.12	0.09	0.01	-	-
23-Mar-92	**+19**	0.18	0.18	0.11	0.06	0.18	0.14	0.08	0.16	0.14	0.02	0.06	0.07	0.12	0.05	0.05	0.04	0.09	0.15	0.08	0.01	-	-
30-Mar-92	**+20**	0.15	0.20	0.09	0.12	0.12	0.14	0.19	0.11	0.14	0.01	0.11	0.08	0.11	0.09	0.07	0.09	0.14	0.12	0.10	0.01	11.5	0.0281
06-Apr-92	+21	0.07	0.10	0.02	0.16	0.18	0.19	0.21	0.06	0.13	0.02	0.12	0.03	0.03	0.04	0.09	0.05	0.13	0.14	0.08	0.01	19.0	0.1949
13-Apr-92	+22	0.28	0.17	0.09	0.14	0.06	0.13	0.12	0.15	0.14	0.02	0.14	0.07	0.13	0.06	0.05	0.06	0.11	0.10	0.09	0.01	14.0	0.0650
20-Apr-92	+23	0.27	0.06	0.13	0.16	0.09	0.12	0.06	0.07	0.12	0.02	0.05	0.04	0.11	0.03	0.03	0.09	0.15	0.11	0.08	0.02	17.5	0.1304
27-Apr-92	+24	0.41	0.03	0.04	0.10	0.04	0.14	0.03	0.02	0.10	0.04	0.04	0.09	0.09	0.01	0.03	0.05	0.13	0.08	0.06	0.01	32.0	1.0409
04-May-92	+25	0.18	0.04	0.03	0.04	0.03	0.13	0.25	0.10	0.10	0.03	0.08	0.09	0.08	0.02	0.06	0.08	0.11	0.08	0.07	0.01	29.0	0.7984
11-May-92	+26	0.11	0.04	0.07	0.06	0.12	0.07	0.32	0.08	0.11	0.03	0.09	0.04	0.14	0.03	0.08	0.07	0.11	0.07	0.08	0.01	-	-
18-May-92	+27	0.08	0.03	0.10	0.05	0.09	0.08	0.05	0.22	0.09	0.02	0.12	0.08	0.13	0.05	0.03	0.04	0.04	0.06	0.07	0.01	-	-
25-May-92	+28	0.27	0.08	0.04	0.04	0.03	0.14	0.07	0.24	0.11	0.03	0.10	0.05	0.16	0.04	0.01	0.09	0.11	0.12	0.09	0.02	-	-
01-Jun-92	+29	0.05	0.04	0.03	0.05	0.03	0.16	0.05	0.39	0.10	0.04	0.08	0.07	0.15	0.19	0.04	0.06	0.12	0.09	0.10	0.02	-	-
08-Jun-92	+30	0.03	0.07	0.01	0.04	0.02	0.13	0.06	0.14	0.06	0.02	0.04	0.03	0.12	0.05	0.03	0.03	0.06	0.15	0.06	0.02	30.5	0.8785
15-Jun-92	+31	0.13	0.08	0.04	0.04	0.12	0.28	0.10	0.16	0.12	0.03	0.10	0.08	0.04	0.05	0.12	0.04	0.17	0.14	0.09	0.02	-	-
22-Jun-92	+32	0.13	0.10	0.04	0.02	0.03	0.03	0.03	0.21	0.07	0.02	0.02	0.04	0.13	0.03	0.04	0.13	0.19	0.04	0.08	0.02	-	-
29-Jun-92	+33	0.02	0.03	0.02	0.08	0.06	0.06	0.03	0.20	0.06	0.02	0.03	0.04	0.09	0.06	0.05	0.11	0.13	0.03	0.07	0.01	-	-
06-Jul-92	+34	0.09	0.03	0.07	0.07	0.11	0.13	0.05	0.40	0.12	0.04	0.05	0.08	0.12	0.09	0.08	0.14	0.11	0.11	0.10	0.01	-	-
13-Jul-92	+35	0.14	0.04	0.12	0.06	0.07	0.05	0.05	0.13	0.08	0.01	0.04	0.15	0.09	0.03	0.01	0.19	0.18	0.16	0.11	0.02	26.5	0.5737

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; weeks printed in bold faces indicate the period when the median values of the two groups were significantly different.

Appendix Table 8.5.14: Serum Total Protein (g/l) of Buffaloes
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	66.6	65.2	62.1	61.0	67.3	71.6	66.6	67.3	65.96	1.09	65.2	60.0	63	56.4	65.2	61.0	76.8	67.8	64.40	2.03	22.5	0.3282
14-Oct-91	-4	68.1	63.4	64.1	58.7	66.3	70.6	66.3	68.6	65.76	1.22	63.8	65.9	61	54.6	70.8	63.4	67.7	65.0	64.01	1.60	-	-
21-Oct-91	-3	68.1	63.4	64.1	58.7	66.3	70.3	66.3	68.6	65.73	1.20	63.8	65.9	61	58.8	63.4	67.7	65.0	63.4	63.61	0.92	-	-
28-Oct-91	-2	65.4	64.5	60.0	60.7	61.9	65.3	65.6	67.6	63.88	0.89	65.1	65.1	64.2	60.7	53.6	65.7	67.0	60.8	62.78	1.43	-	-
04-Nov-91	-1	65.0	63.4	58.6	64.4	65.6	62.6	60.1	61.5	62.65	0.81	62.9	64.3	64.3	64.6	57.6	65.6	61.2	61.3	62.73	0.86	-	-
11-Nov-91	0	62.6	63.4	62.2	65.8	62.5	66.3	61.7	66.9	63.93	0.68	64.1	61.9	63.5	69.2	62.2	65.3	65.0	63.5	64.34	0.76	28.5	0.7209
18-Nov-91	+1	65.4	60.3	63.2	65.1	67.9	65.6	65.0	65.3	64.73	0.73	65.7	59.4	65.0	66.5	60.0	61.5	68.1	55.6	62.73	1.41	-	-
25-Nov-91	+2	65.0	65.9	64.7	63.9	62.3	63.7	61.0	62.9	63.68	0.52	62.8	57.9	65.1	65.1	63.7	65.1	61.5	56.4	62.20	1.12	-	-
02-Dec-91	+3	62.9	64.2	66.8	65.1	61.3	63.9	61.2	64.9	63.79	0.64	60.0	63.8	63.8	61.2	61.8	65.7	62.1	59.5	62.24	0.69	-	-
09-Dec-91	+4	62.5	63.7	65.6	66.5	65.3	69.6	66.8	66.5	65.81	0.71	65.2	61.4	65.4	60.0	63.7	66.1	65.9	66.5	64.28	0.79	-	-
16-Dec-91	+5	63.9	62.0	62.2	62.9	70.3	69.2	67.1	67.9	65.69	1.10	64.3	65.0	59.6	63.3	62.6	62.4	68.8	61.6	63.45	0.90	21.0	0.2786
23-Dec-91	+6	67.7	67.0	68.3	64.1	66.6	69.4	70.4	67.5	67.63	0.63	62.2	64.1	62.6	65.4	64.5	67.2	69.3	64.1	64.93	0.78	-	-
30-Dec-91	+7	66.6	65.9	66.1	63.1	64.3	68.1	69.4	65.3	66.10	0.66	65.2	60.8	62.5	63.3	62.4	64.3	68.0	60.5	63.38	0.81	-	-
06-Jan-92	+8	65.4	67.6	67.9	63.1	71.6	69.1	71.0	71.8	68.44	1.02	64.2	63.3	60.7	62.7	63.6	63.4	65.6	63.6	63.39	0.46	-	-
13-Jan-92	+9	64.7	69.4	68.2	64.3	68.7	68.6	75.2	69.2	68.54	1.11	67.6	64.7	60.0	61.4	61.0	64.7	65.5	66.3	63.90	0.91	-	-
20-Jan-92	+10	66.3	68.7	69.1	63.7	66.1	60.7	68.5	66.9	66.25	0.94	65.5	65.4	62.7	64.5	65.9	69.5	62.4	60.0	64.49	0.94	18.0	0.1605
27-Jan-92	+11	67.8	69.1	67.1	62.2	67.4	62.2	66.9	66.9	66.21	0.85	67.1	64.4	60.1	64.8	62.6	65.4	70.8	63.7	64.87	1.04	-	-
03-Feb-92	+12	72.3	73.7	72.0	62.0	77.5	64.6	69.2	75.5	70.86	1.76	67.6	67.8	60.4	63.8	61.8	71.0	69.0	73.7	66.88	1.51	-	-
10-Feb-92	+13	83.2	78.6	73.9	67.8	69.5	64.2	68.1	73.7	72.38	2.07	67.0	67.4	59.9	62.4	61.0	71.5	58.7	67.4	64.43	1.50	-	-
17-Feb-92	+14	70.0	71.3	77.5	62.8	73.4	63.8	70.9	70.6	70.05	1.58	64.4	63.4	58.0	64.0	62.5	72.7	68.4	69.2	65.33	1.51	-	-
24-Feb-92	+15	63.9	67.1	71.0	63.0	75.9	62.4	67.6	72.9	67.96	1.63	69.9	66.7	60.0	65.0	63.9	69.5	82.2	65.9	67.89	2.18	29.5	0.7984
02-Mar-92	+16	71.1	65.4	65.0	66.6	72.7	68.0	64.1	72.3	68.15	1.14	71.1	67.7	60.2	67.1	61.6	78.9	74.3	65.7	68.33	2.08	-	-
09-Mar-92	+17	66.4	65.8	67.0	60.7	71.4	64.4	69.5	75.5	67.58	1.50	65.6	68.1	60.1	62.1	60.3	65.0	68.7	65.4	64.40	1.09	-	-
16-Mar-92	+18	64.4	60.9	63.4	59.7	64.6	58.1	66.5	69.3	63.36	1.22	67.5	65.2	62.9	64.8	60.1	60.3	67.7	61.7	63.79	0.99	-	-
23-Mar-92	+19	58.4	63.2	68.9	62.0	63.3	57.6	67.4	68.5	63.66	1.44	65.6	66.4	60.5	63.2	63.3	58.8	68.7	67.0	64.19	1.12	30.0	0.8785
30-Mar-92	**+20**	54.4	59.5	62.8	60.6	49.6	58.1	68.8	64.5	59.78	1.97	64.3	66.6	63.5	65.1	63.7	65.1	67.8	64.1	65.03	0.49	12.0	0.0379
06-Apr-92	**+21**	51.1	58.6	63.5	61.4	60.7	56.8	68.3	60.1	60.07	1.66	62.6	66.0	62.5	60.7	65.8	65.7	65.7	63.8	64.09	0.66	-	-
13-Apr-92	**+22**	51.2	60.3	61.1	56.1	59.7	56.1	57.2	64.6	58.29	1.34	64.9	66.7	65.4	60.5	62.1	61.5	66.5	60.8	63.54	0.86	-	-
20-Apr-92	**+23**	47.1	48.3	56.2	48.6	53.4	49.7	64.3	60.2	53.46	2.07	60.2	65.4	65.6	65.4	65.9	65.3	65.8	62.5	64.51	0.68	-	-
27-Apr-92	**+24**	51.6	54.9	67.7	51.6	56.0	56.7	60.0	61.5	57.50	1.79	65.9	62.0	58.3	61.6	62.4	65.9	66.8	66.8	63.72	1.03	-	-
04-May-92	**+25**	50.1	55.5	63.7	54.4	58.3	53.6	62.9	57.8	57.05	1.53	61.6	61.6	62.4	58.9	61.2	62.9	64.9	65.9	62.43	0.72	11.5	0.0281
11-May-92	**+26**	52.3	53.7	56.5	64.5	60.3	54.7	56.7	58.3	57.13	1.29	64.9	61.5	59.4	62.9	60.0	65.3	61.2	65.5	62.59	0.81	7.0	0.0070
18-May-92	**+27**	49.6	48.9	57.8	52.0	55.9	52.2	57.3	57.4	53.88	1.20	63.0	60.0	61.3	63.7	62.4	62.9	63.4	60.7	62.18	0.45	0.0	0.0002
25-May-92	**+28**	47.3	49.0	58.0	53.7	55.1	49.3	57.0	63.4	54.10	1.80	65.9	62.5	59.9	63.5	62.5	65.7	60.7	61.7	62.80	0.72	5.0	0.0030
01-Jun-92	**+29**	37.0	45.3	56.5	50.0	55.2	49.1	55.2	53.8	50.27	2.18	61.2	63.2	60.7	61.7	59.7	63.4	62.2	65.4	62.19	0.59	0.0	0.0002
08-Jun-92	**+30**	52.7	49.7	62.4	55.6	56.2	53.5	62.6	61.3	56.75	1.61	57.3	61.2	51.7	58.4	61.6	62.8	66.4	62.0	60.18	1.45	20.0	0.2345
15-Jun-92	**+31**	50.1	53.1	64.9	53.3	58.8	56.9	62.3	60.9	57.54	1.69	61.7	63.5	54.9	57.3	60.7	53.1	64.2	64.9	60.04	1.47	-	-
22-Jun-92	**+32**	49.2	50.7	56.7	56.0	54.0	49.5	50.7	52.7	52.42	0.96	52.8	52.8	65.5	55.7	57.3	51.2	60.0	60.5	56.98	1.60	-	-
29-Jun-92	**+33**	53.3	57.4	62.8	58.2	63.9	58.9	60.4	64.5	59.93	1.25	58.0	60.1	55.5	51.5	61.0	61.7	60.1	60.3	58.55	1.14	-	-
06-Jul-92	**+34**	52.9	54.3	66.6	57.3	57.7	58.7	56.1	69.3	59.12	1.92	58.7	58.0	57.7	55.6	66.6	59.3	62.6	59.1	59.69	1.13	-	-
13-Jul-92	**+35**	48.9	47.8	59.4	51.2	55.9	61.4	61.2	66.8	56.57	2.25	62.9	61.7	57.0	57.7	61.2	61.0	65.2	61.0	60.97	0.88	19.5	0.1949

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; weeks printed in bold faces indicate the period when the median values of the two groups were significantly different.

Appendix Table 8.5.15: Serum Albumun Levels (g/l) of Buffaloes
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	30.2	33.4	33.5	32.5	34.1	30.7	30.7	31.7	32.10	0.49	31.9	33.8	31.0	31.2	33.9	32.7	32.5	32.8	32.48	0.36	25.5	0.5054
14-Oct-91	-4	32.7	32.8	33.2	32.0	32.4	31.9	30.1	30.6	31.96	0.36	32.3	32.8	30	32.5	32.6	33.7	32.6	31.9	32.35	0.31	-	-
21-Oct-91	-3	32.0	32.8	32.2	31.0	32.4	31.9	30.1	30.6	31.63	0.31	32.3	32.8	30	33.5	33.6	33.7	32.6	30.9	32.48	0.41	-	-
28-Oct-91	-2	33.3	32.1	32.0	31.6	33.1	33.1	33.1	32.5	32.60	0.21	34.0	31.8	31.7	32.7	33.4	32.2	33.1	31.7	32.58	0.29	-	-
04-Nov-91	-1	34.2	32.9	30.1	30.6	33.5	33.5	31.6	32.2	32.33	0.49	34.7	33.1	31.0	32.5	34.2	32.3	31.3	31.7	32.60	0.44	-	-
11-Nov-91	0	33.1	32.6	30.9	32.5	32.2	32.2	33.6	32.5	32.45	0.26	33.7	35.5	30.9	31.9	35.0	32.4	32.6	32.0	33.00	0.53	29.0	0.7984
18-Nov-91	+1	32.9	30.0	32.1	33.6	33.2	30.4	35.2	33.8	32.65	0.58	30.2	34.2	32.6	30.0	34.2	30.6	32.9	32.7	32.18	0.56	-	-
25-Nov-91	+2	34.7	31.5	31.2	32.1	32.4	32.0	34.1	35.3	32.91	0.52	32.1	35.6	30.8	32.1	32.7	30.0	30.5	32.4	32.03	0.58	-	-
02-Dec-91	+3	36.2	34.1	32.7	32.0	30.2	31.8	36.4	35.6	33.63	0.76	35.4	36.7	32.7	32.7	31.3	31.8	30.3	33.2	33.01	0.70	-	-
09-Dec-91	+4	36.1	36.3	31.7	34.8	32.7	37.0	38.5	35.1	35.28	0.74	33.3	37.9	34.4	31.7	34.1	33.8	37.5	34.8	34.69	0.69	-	-
16-Dec-91	+5	32.1	31.8	32.2	33.3	30.8	34.2	36.1	36.6	33.39	0.69	30.9	32.4	35.4	32.4	37.5	30.6	34.0	30.4	32.95	0.84	28.0	0.7209
23-Dec-91	+6	35.8	30.8	31.7	33.7	30.9	32.7	38.0	36.1	33.71	0.88	31.0	30.7	33.7	33.4	38.0	36.0	32.5	30.8	33.26	0.87	-	-
30-Dec-91	+7	33.9	35.6	35.6	39.8	35.4	35.9	38.7	31.2	35.76	0.88	36.6	34.6	35.7	33.9	38.0	34.6	37.5	33.5	35.55	0.55	-	-
06-Jan-92	+8	34.2	32.5	32.9	34.8	35.7	32.6	39.8	34.9	34.68	0.79	33.6	32.9	37.8	32.4	35.0	38.3	34.6	39.3	35.49	0.87	-	-
13-Jan-92	+9	32.6	31.5	32.0	32.8	33.7	32.5	35.3	31.8	32.78	0.41	32.3	32.4	33.4	32.3	36.2	33.3	36.0	32.9	33.60	0.53	-	-
20-Jan-92	+10	33.0	34.9	30.0	33.1	31.8	30.9	33.7	33.5	32.61	0.53	33.7	33.1	33.4	30.1	32.0	37.3	36.9	33.8	33.78	0.79	21.0	0.2786
27-Jan-92	+11	32.3	34.1	34.3	33.9	34.8	35.0	36.2	33.3	34.24	0.38	37.0	34.6	35.8	35.0	37.5	36.9	37.3	34.1	36.03	0.44	-	-
03-Feb-92	+12	33.4	32.3	34.6	34.3	33.5	33.5	34.3	32.6	33.56	0.27	35.5	35.3	34.9	33.2	35.8	38.2	37.2	35.9	35.75	0.49	-	-
10-Feb-92	+13	39.3	35.5	31.4	32.1	26.2	35.3	39.1	34.2	34.15	1.42	34.9	32.5	36.4	34.0	35.6	36.9	35.2	37.6	35.40	0.55	-	-
17-Feb-92	+14	34.0	33.8	33.0	34.6	33.9	31.2	33.4	34.6	33.55	0.36	35.0	32.8	33.3	32.0	35.3	36.1	41.7	34.5	35.10	0.99	-	-
24-Feb-92	+15	35.5	30.7	31.6	33.5	32.2	34.5	34.9	33.6	33.32	0.56	30.5	35.1	33.6	36.0	34.3	37.0	36.4	37.3	35.03	0.73	15.5	0.0830
02-Mar-92	+16	33.7	30.4	30.0	36.3	33.4	36.3	35.6	35.4	33.89	0.83	33.1	36.3	35.2	38.1	35.4	38.0	36.5	37.8	36.29	0.57	-	-
09-Mar-92	+17	34.6	29.5	27.9	32.4	31.1	35.6	38.9	36.7	33.34	1.24	35.0	35.9	39.8	36.2	37.2	35.8	35.7	38.6	36.78	0.55	-	-
16-Mar-92	+18	35.0	31.8	31.3	33.3	34.1	34.2	36.0	32.1	33.47	0.55	37.3	33.8	35.2	36.5	36.4	34.6	35.9	36.0	35.71	0.37	-	-
23-Mar-92	-19	33.1	33.3	28.5	36.0	29.4	29.8	37.3	35.4	32.85	1.09	35.9	33.3	38.1	34.8	36.3	35.0	39.2	38.9	36.42	0.70	-	-
30-Mar-92	+20	34.4	28.3	26.1	33.8	29.1	30.2	37.4	32.7	31.49	1.23	33.4	33.9	34.2	32.0	36.3	35.8	38.1	35.4	34.89	0.63	14.0	0.0650
06-Apr-92	**+21**	28.4	29.8	26.2	32.9	29.9	31.2	33.9	32.8	30.63	0.86	32.6	32.9	32.2	33.1	35.7	33.9	36.5	34.6	33.94	0.51	9.0	0.0148
13-Apr-92	**+22**	27.2	29.9	28.7	30.3	29.3	30.6	32.0	33.7	30.23	0.65	31.0	33.3	31.9	32.9	36.9	35.7	37.2	34.4	34.17	0.76	6.0	0.0047
20-Apr-92	**+23**	26.2	25.8	28.4	27.9	30.6	30.1	33.2	29.9	29.02	0.81	37.3	35.0	32.9	33.2	32.6	32.5	35.2	32.9	33.95	0.56	4.5	0.0019
27-Apr-92	**+24**	28.6	28.3	27.9	30.5	29.1	30.1	34.1	31.2	29.98	0.67	33.1	32.2	32.3	33.1	31.9	31.2	32.7	32.5	32.38	0.21	8.5	0.0104
04-May-92	**+25**	27.8	25.7	27.2	27.7	25.3	29.1	31.6	30.0	28.05	0.70	30.2	30.9	32.1	29.3	31.0	30.9	32.2	31.0	30.96	0.32	7.0	0.0070
11-May-92	**+26**	27.7	29.4	29.2	30.7	29.5	27.7	31.6	30.8	29.58	0.47	33.0	34.2	30.3	31.7	34.1	30.0	31.9	34.8	32.50	0.61	-	-
18-May-92	**+27**	28.4	25.8	28.5	28.3	28.9	22.6	29.7	28.6	27.60	0.77	31.4	30.5	30.7	31.9	31.3	32.0	32.2	29.8	31.23	0.28	-	-
25-May-92	**+28**	29.5	29.1	32.4	30.9	27.1	28.0	32.9	30.0	29.99	0.67	33.1	32.5	33.4	31.4	34.0	33.5	33.2	31.1	32.78	0.34	-	-
01-Jun-92	**+29**	28.7	27.0	31.1	30.2	27.8	28.7	31.7	31.1	29.54	0.57	34.7	34.8	31.0	31.2	33.1	36.2	34.2	29.3	33.05	0.78	-	-
08-Jun-92	**+30**	26.2	33.9	31.9	29.3	27.2	30.7	33.4	31.5	30.51	0.92	35.4	32.3	30.4	34.0	38.3	35.2	35.8	35.7	34.65	0.80	7.0	0.0030
15-Jun-92	**+31**	25.5	31.6	29.0	34.2	28.6	29.3	31.5	32.7	30.30	0.91	37.1	31.7	30.1	31.2	33.3	35.1	35.1	34.1	33.46	0.77	-	-
22-Jun-92	**+32**	25.8	28.4	26.8	29.4	27.7	30.3	28.6	31.9	28.60	0.64	35.3	30.6	30.9	30.4	34.6	32.3	32.9	30.7	32.20	0.63	-	-
29-Jun-92	**+33**	27.5	30.8	29.1	33.9	29.9	31.2	30.6	32.1	30.64	0.64	33.6	33.8	31.2	32.7	34.8	34.1	35.5	33.3	33.62	0.43	-	-
06-Jul-92	**+34**	26.9	31.7	33.6	32.2	31.0	29.8	31.5	34.9	31.44	0.79	32.0	34.7	30.9	33.9	34.9	36.6	36.5	38.3	34.72	0.81	-	-
13-Jul-92	**+35**	24.5	30.4	32.6	31.2	30.3	30.3	33.7	32.6	30.71	0.93	34.9	34.2	33.5	31.8	33.6	33.9	37.0	34.7	34.19	0.49	5.0	0.0030

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; weeks printed in bold faces indicate the period when the median values of the two groups were significantly different.

Appendix Table 8.5.16: Serum Globulin (g/l) of Buffaloes
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	36.4	31.8	28.6	28.5	33.2	40.9	35.9	35.6	33.86	1.39	33.3	26.2	31.8	25.2	31.3	28.3	44.3	35.0	31.93	2.00	20.5	0.2345
14-Oct-91	-4	35.4	30.6	30.9	26.7	33.9	38.7	36.2	38.0	33.80	1.36	31.5	33.1	30.5	22.1	38.2	29.7	35.1	33.1	31.66	1.56	-	-
21-Oct-91	-3	36.1	30.6	31.9	27.7	33.9	38.4	36.2	38.0	34.10	1.25	31.5	33.1	30.5	25.3	29.8	34.0	32.4	32.5	31.14	0.90	-	-
28-Oct-91	-2	32.1	32.4	28.0	29.1	28.8	32.2	32.5	35.1	31.28	0.80	31.1	33.3	32.5	28.0	20.2	33.5	33.9	29.1	30.20	1.51	-	-
04-Nov-91	-1	30.8	30.5	28.5	33.8	32.1	29.1	28.5	29.3	30.33	0.62	28.2	31.2	33.3	32.1	23.4	33.3	29.9	29.6	30.13	1.08	-	-
11-Nov-91	0	29.5	30.8	31.3	33.3	30.3	34.1	28.1	34.4	31.48	0.75	30.4	26.4	32.6	37.3	27.2	32.9	32.4	31.5	31.34	1.14	31.0	0.9591
18-Nov-91	+1	32.5	30.3	31.1	31.5	34.7	35.2	29.8	31.5	32.08	0.65	35.5	25.2	32.4	36.5	25.8	30.9	35.2	22.9	30.55	1.74	-	-
25-Nov-91	+2	30.3	34.4	33.5	31.8	29.9	31.7	26.9	27.6	30.76	0.87	30.7	22.3	34.3	33.0	31.0	35.1	31.0	24.0	30.18	1.54	-	-
02-Dec-91	+3	26.7	30.1	34.1	33.1	31.1	32.1	24.8	29.3	30.16	1.05	24.6	27.1	31.1	28.5	30.5	33.9	31.8	26.3	29.23	1.04	-	-
09-Dec-91	+4	26.4	27.4	33.9	31.7	32.6	32.6	28.3	31.4	30.54	0.92	31.9	23.5	31.0	28.3	29.6	32.3	28.4	31.7	29.59	0.96	-	-
16-Dec-91	+5	31.8	30.2	30.0	29.6	39.5	35.0	31.0	31.3	32.30	1.11	33.4	32.6	24.2	30.9	25.1	31.8	34.8	31.2	30.50	1.27	30.5	0.8785
23-Dec-91	+6	31.9	36.2	36.6	30.4	35.7	36.7	32.4	31.4	33.91	0.87	31.2	33.4	28.9	32.0	26.5	31.2	36.8	33.3	31.67	1.02	21.0	0.2786
30-Dec-91	+7	32.7	30.3	30.5	23.3	28.9	32.2	30.7	34.1	30.34	1.08	28.6	26.2	26.8	29.4	24.4	29.7	30.5	27.0	27.83	0.68	12.5	0.0379
06-Jan-92	**+8**	31.2	35.1	35.0	28.3	35.9	36.5	31.2	36.9	33.76	1.03	30.6	30.4	22.9	30.3	28.6	25.1	31.0	24.3	27.90	1.08	5.0	0.0030
13-Jan-92	**+9**	32.1	37.9	36.2	31.5	35.0	36.1	39.9	37.4	35.76	0.94	35.3	32.3	26.6	29.1	24.8	31.4	29.5	33.4	30.30	1.16	7.0	0.0070
20-Jan-92	**+10**	33.3	33.8	39.1	30.6	34.3	34.8	34.8	33.4	34.26	0.78	31.8	32.3	29.3	28.5	29.9	32.2	25.5	26.2	29.46	0.87	3.0	0.0011
27-Jan-92	**+11**	35.5	35.0	32.7	32.3	36.6	32.2	30.7	33.6	33.59	0.66	30.1	29.8	24.4	29.8	25.1	28.5	33.5	29.6	28.84	0.97	8.0	0.0104
03-Feb-92	**+12**	38.9	41.4	37.4	27.7	44.0	31.1	34.9	42.9	37.29	1.91	32.1	32.5	25.5	30.6	26.0	32.8	31.8	37.8	31.13	1.30	13.0	0.0499
10-Feb-92	**+13**	43.9	43.0	42.5	35.8	43.2	28.9	28.9	39.6	38.23	2.10	32.2	35.0	23.5	28.4	25.3	34.6	23.5	29.8	29.03	1.54	8.0	0.0104
17-Feb-92	**+14**	35.9	37.6	44.5	28.3	39.6	32.6	37.5	36.0	36.50	1.58	29.4	30.6	24.7	32.0	27.1	36.5	26.7	34.7	30.22	1.35	9.0	0.0148
24-Feb-92	**+15**	28.4	36.4	39.4	29.5	43.7	27.9	32.7	39.3	34.65	1.95	39.3	31.6	26.4	29.0	29.6	32.5	45.8	28.7	32.87	2.15	27.0	0.6454
02-Mar-92	+16	37.4	35.0	35.0	30.4	39.3	31.7	28.5	36.9	34.26	1.24	38.0	31.3	25.0	29.0	26.3	41.0	37.8	27.9	32.03	2.01	-	-
09-Mar-92	+17	31.8	36.3	33.1	28.3	36.3	28.8	30.6	38.8	33.00	1.26	30.6	32.2	28.3	27.9	29.0	29.2	33.0	26.8	29.61	0.71	-	-
16-Mar-92	+18	29.4	29.1	32.1	26.4	30.5	23.8	30.5	37.2	29.89	1.31	30.2	31.5	27.7	28.3	23.7	25.7	31.8	25.7	28.08	0.97	-	-
23-Mar-92	+19	25.3	29.9	40.4	26.0	34.0	27.8	30.0	33.1	30.81	1.63	29.8	33.1	22.4	28.4	27.0	23.8	29.5	28.1	27.76	1.13	-	-
30-Mar-92	+20	20.0	31.2	36.7	26.8	20.5	27.9	31.4	31.8	28.29	1.90	30.9	32.7	29.3	33.1	27.4	29.3	29.7	28.7	30.14	0.65	27.0	0.6454
06-Apr-92	+21	22.7	28.8	37.4	28.5	30.8	25.6	34.5	27.3	29.45	1.56	30.0	33.1	30.3	27.6	30.1	31.8	29.2	29.2	30.15	0.56	-	-
13-Apr-92	+22	24.0	30.4	32.4	25.8	30.3	25.5	25.2	30.9	28.06	1.08	33.9	33.4	33.5	27.6	25.2	25.8	29.3	26.5	29.37	1.22	-	-
20-Apr-92	+23	26.9	22.5	27.7	20.8	22.8	29.5	31.1	30.3	26.44	1.30	22.9	30.4	32.7	32.2	33.3	32.8	30.6	29.6	30.55	1.11	-	-
27-Apr-92	+24	23.0	26.6	39.8	21.1	26.9	26.6	25.9	30.3	27.52	1.87	32.8	29.8	26.0	28.5	30.5	34.7	34.1	34.3	31.34	1.05	-	-
04-May-92	+25	22.3	29.8	36.6	26.8	33.0	24.5	31.4	27.8	29.00	1.54	31.4	30.8	30.3	29.7	30.1	32.0	32.6	34.9	31.47	0.57	20.0	0.2345
11-May-92	+26	24.7	24.3	27.3	33.8	30.8	26.9	25.1	27.5	27.55	1.08	31.9	27.3	29.1	31.2	25.8	35.3	29.3	30.7	30.09	0.97	-	-
18-May-92	+27	21.2	23.2	31.3	23.7	29.0	29.6	30.5	31.8	27.53	1.38	31.6	29.5	30.6	31.8	31.1	30.9	31.2	30.9	30.95	0.23	-	-
25-May-92	+28	27.8	29.9	25.6	22.8	28.0	21.3	24.1	33.4	26.61	1.31	32.8	30.0	26.5	32.1	28.5	32.2	27.5	30.6	30.02	0.77	-	-
01-Jun-92	+29	28.3	28.2	25.4	29.8	27.4	20.4	23.5	22.7	25.72	1.09	26.5	28.4	29.7	30.5	26.6	27.2	28.0	36.1	29.14	1.04	-	-
08-Jun-92	+30	26.5	25.7	30.6	26.3	29.0	22.8	29.2	29.8	27.49	0.86	31.9	28.9	27.3	28.4	30.3	27.6	30.7	26.3	28.91	0.63	23.0	0.3823
15-Jun-92	+31	24.7	21.5	35.9	29.1	30.2	27.6	30.8	28.2	28.49	1.41	29.7	31.8	32.8	26.1	27.4	28.0	29.1	30.8	29.46	0.75	-	-
22-Jun-92	+32	23.4	24.3	31.8	27.6	26.3	24.0	22.1	20.8	25.04	1.16	27.5	32.2	34.6	25.3	22.8	28.8	27.1	29.8	28.53	1.24	-	-
29-Jun-92	+33	25.8	26.6	33.7	24.3	34.0	27.7	29.8	32.5	29.29	1.25	24.4	26.3	29.2	28.9	26.2	27.6	24.6	27.1	26.80	0.59	-	-
06-Jul-92	+34	26.0	22.6	33.0	25.2	26.7	29.0	24.6	34.4	27.68	1.37	26.7	30.4	26.8	32.6	31.7	32.6	26.1	28.8	29.47	0.90	-	-
13-Jul-92	+35	24.3	27.5	26.8	30.0	25.6	31.1	27.5	34.2	28.37	1.06	28.0	27.5	32.5	25.9	27.6	27.1	28.2	26.4	27.90	0.67	30.0	0.8785

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; weeks printed in bold faces indicate the period when the median values of the two groups were significantly different.

Appendix Table 8.5.17: Serum Albumun to Globulin Ratio of Buffaloes
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	0.8	1.1	1.2	1.1	1	0.8	0.9	0.9	0.96	0.05	1	1.3	1	1.2	1.1	1.2	0.7	0.9	1.05	0.06	23.0	0.3823
14-Oct-91	-4	0.9	1.1	1.1	1.2	1	0.8	0.8	0.8	0.96	0.05	1	1	1	1.5	0.9	1.1	0.9	1	1.05	0.06	-	-
21-Oct-91	-3	0.9	1.1	1	1.1	1	0.8	0.8	0.8	0.94	0.04	1	1	1	1.3	1.1	1	1	1	1.05	0.04	-	-
28-Oct-91	-2	1	1	1.1	1.1	1.1	1	1	0.9	1.05	0.03	1.1	1	1	1.2	1.7	1	1	1.1	1.11	0.08	-	-
04-Nov-91	-1	1.1	1.1	1.1	0.9	1	1.2	1.1	1.1	1.07	0.02	1.2	1.1	0.9	1	1.5	1	1	1.1	1.10	0.06	-	-
11-Nov-91	0	1.1	1.1	1	1	1.1	0.9	1.2	0.9	1.04	0.03	1.1	1.3	0.9	0.9	1.3	1	1	1	1.07	0.06	29.0	0.7984
18-Nov-91	+1	1	1	1	1.1	1	0.9	1.2	1.1	1.02	0.03	0.9	1.4	1	0.8	1.3	1	0.9	1.4	1.09	0.08	-	-
25-Nov-91	+2	1.1	0.9	0.9	1	1.1	1	1.3	1.3	1.08	0.05	1	1.6	0.9	1	1.1	0.9	1	1.4	1.09	0.08	-	-
02-Dec-91	+3	1.4	1.1	1	1	1	1	1.5	1.2	1.13	0.07	1.4	1.4	1.1	1.1	1	0.9	1	1.3	1.15	0.06	-	-
09-Dec-91	+4	1.4	1.3	0.9	1.1	1	1.1	1.4	1.1	1.17	0.05	1	1.6	1.1	1.1	1.2	1	1.3	1.1	1.19	0.06	-	-
16-Dec-91	+5	1	1.1	1.1	1.1	0.8	1	1.2	1.2	1.04	0.04	0.9	1	1.5	1	1.5	1	1	1	1.10	0.08	25.0	0.5054
23-Dec-91	+6	1.1	0.9	0.9	1.1	0.9	0.9	1.2	1.1	1.00	0.05	1	0.9	1.2	1	1.4	1.2	0.9	0.9	1.06	0.06	-	-
30-Dec-91	+7	1	1.2	1.2	1.7	1.2	1.1	1.3	0.9	1.20	0.08	1.3	1.3	1.3	1.2	1.6	1.2	1.2	1.2	1.28	0.04	18.0	0.1605
06-Jan-92	+8	1.1	0.9	0.9	1.2	1	0.9	1.3	0.9	1.04	0.05	1.1	1.1	1.7	1.1	1.2	1.5	1.1	1.6	1.30	0.08	12.0	0.0379
13-Jan-92	**+9**	1	0.8	0.9	1	1	0.9	0.9	0.9	0.92	0.03	0.9	1	1.3	1.1	1.5	1.1	1.2	1	1.13	0.06	7.0	0.0070
20-Jan-92	**+10**	1	1	0.8	1.1	0.9	1	1	1	0.98	0.03	1.1	1	1.1	1.2	1.4	1.2	1.4	1.3	1.22	0.05	4.0	0.0019
27-Jan-92	**+11**	0.9	1	1	1	1.1	1	1.2	1	1.03	0.03	1.2	1.2	1.3	1.2	1.2	1	1.1	1.2	1.17	0.03	7.0	0.0070
03-Feb-92	**+12**	0.9	0.8	0.9	1.2	0.8	1.1	1	0.8	0.92	0.06	1.1	1.1	1.4	1.1	1.4	1.2	1.2	0.9	1.16	0.05	8.0	0.0104
10-Feb-92	**+13**	0.9	0.8	0.7	0.9	0.6	1.2	1.4	0.9	0.93	0.08	1.1	0.9	1.5	1.2	1.4	1.1	1.5	1.3	1.25	0.07	9.0	0.0148
17-Feb-92	**+14**	0.9	0.9	0.7	1.2	0.9	1	0.9	1	0.93	0.05	1.2	1.1	1.4	1	1.3	1	1.6	1	1.18	0.07	5.0	0.0030
24-Feb-92	**+15**	1.3	0.8	0.8	1.1	0.7	1.2	1.1	0.9	0.99	0.07	1.1	1.1	1.3	1.2	1.2	1.1	1.2	1.3	1.20	0.02	11.0	0.0281
02-Mar-92	+16	0.9	0.9	0.9	1.2	0.8	1.1	1.2	1	1.00	0.05	0.9	1.2	1.4	1.3	1.3	0.9	1	1.4	1.17	0.07	14.0	0.0650
09-Mar-92	+17	1.1	0.8	0.7	1.1	0.8	1.2	1.3	0.9	1.00	0.07	1.1	1.1	1.2	1.4	1.3	1.2	1.1	1.2	1.22	0.04	15.0	0.0830
16-Mar-92	+18	1.2	1.1	1	1.3	1.1	1.4	1.2	0.9	1.14	0.06	1.2	1.1	1.3	1.3	1.5	1.3	1.1	1.4	1.28	0.05	16.0	0.1049
23-Mar-92	+19	1.3	1.1	0.7	1.4	0.9	1.1	1.2	1.1	1.10	0.07	1.2	1	1.3	1.2	1.3	1.2	1.3	1.4	1.24	0.04	20.0	0.2345
30-Mar-92	+20	1.7	0.9	0.7	1.3	1.4	1.1	1.2	1	1.16	0.10	1.1	1	1.2	1	1.3	1.2	1.3	1.2	1.16	0.04	30.0	0.8785
06-Apr-92	+21	1.3	1	0.7	1.2	1	1.2	1	1.2	1.06	0.06	1.1	1	1.1	1.2	1.2	1.1	1.3	1.2	1.13	0.03	-	-
13-Apr-92	+22	1.1	1	0.9	1.2	1	1.2	1.3	1.1	1.09	0.04	0.9	1	1	1.2	1.5	1.4	1.3	1.3	1.19	0.07	-	-
20-Apr-92	+23	1	1.1	1	1.3	1.3	1	1.1	1	1.11	0.05	1.6	1.2	1	1	1	1	1.2	1.1	1.13	0.07	-	-
27-Apr-92	+24	1.2	1.1	0.7	1.4	1.1	1.1	1.3	1	1.13	0.07	1	1.1	1.2	1.2	1	0.9	1	0.9	1.04	0.04	-	-
04-May-92	+25	1.2	0.9	0.7	1	0.8	1.2	1	1.1	0.99	0.06	1	1	1.1	1	1	1	1	0.9	0.99	0.02	27.0	0.6454
11-May-92	+26	1.1	1.2	1.1	0.9	1	1	1.3	1.1	1.08	0.04	1	1.3	1	1	1.3	0.8	1.1	1.1	1.09	0.05	-	-
18-May-92	+27	1.3	1.1	0.8	1.2	0.9	0.8	0.9	0.8	0.98	0.07	1	1	1	1	1	1	1	1	1.01	0.01	-	-
25-May-92	+28	1.1	1	1.3	1.4	1	1.3	1.4	0.9	1.15	0.06	1	1.1	1.3	1	1.2	1	1.2	1	1.10	0.04	-	-
01-Jun-92	+29	1	1	1.2	1	1	1.4	1.3	1.4	1.17	0.06	1.3	1.2	1	1	1.2	1.3	1.2	0.8	1.15	0.06	-	-
08-Jun-92	+30	1	1.3	1	1.1	0.9	1.3	1.1	1.1	1.12	0.05	1.1	1.1	1.1	1.2	1.3	1.3	1.2	1.4	1.20	0.03	18.0	0.1605
15-Jun-92	+31	1	1.5	0.8	1.2	0.9	1.1	1	1.2	1.08	0.06	1.2	1	0.9	1.2	1.2	1.3	1.2	1.1	1.14	0.04	-	-
22-Jun-92	+32	1.1	1.1	0.8	1	1.1	1.3	1.3	1.5	1.14	0.07	1.3	1	0.9	1.2	1.5	1.1	1.2	1	1.15	0.07	-	-
29-Jun-92	+33	1.1	1.2	0.9	1.4	1	1.1	1	1	1.08	0.05	1.2	1.3	1.1	1.1	1.3	1.2	1.2	1.2	1.22	0.03	-	-
06-Jul-92	+34	1	1.4	1	1.3	1.2	1	1.3	1	1.15	0.05	1.2	1.1	1.2	1	1.1	1.1	1.4	1.3	1.19	0.04	-	-
13-Jul-92	+35	1	1.1	1.2	1.4	1.2	1	1.2	1	1.14	0.05	1.2	1.2	1	1.1	1.2	1.2	1.3	1.3	1.21	0.03	17.0	0.1304

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; weeks printed in bold faces indicate the period when the median values of the two groups were significantly different.

Appendix Table 8.5.18: Serum Glutamate Dehydrogenase Activity (U./l) of Buffaloes Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	16	9	13	10	15	12	10	14	12.3	0.9	14	19	12	15	11	17	12	11	13.9	1.0	23.0	0.3823
14-Oct-91	-4	13	12	11	10	12	18	12	11	12.2	0.8	10	14	14	13	13	16	10	13	12.7	0.6	-	-
21-Oct-91	-3	13	15	10	15	13	10	11	9	11.9	0.7	12	11	11	13	14	14	5	13	11.5	0.9	-	-
28-Oct-91	-2	14	12	13	10	11	14	12	14	12.4	0.5	10	11	12	13	12	17	16	11	12.7	0.8	-	-
04-Nov-91	-1	16	14	11	15	13	18	16	16	14.7	0.7	14	10	16	15	11	19	17	17	14.9	1.0	-	-
11-Nov-91	0	13	8	15	10	10	18	10	11	11.8	1.1	14	14	12	13	13	14	17	16	14.0	0.6	17.0	0.1304
18-Nov-91	+1	14	9	15	12	13	14	17	16	13.7	0.8	10	11	16	11	13	13	16	13	12.7	0.7	23.5	0.3823
25-Nov-91	+2	13	10	13	18	11	12	10	14	12.5	0.9	17	19	14	11	12	16	11	11	13.8	1.0	24.5	0.4418
02-Dec-91	+3	16	14	26	35	11	14	12	20	18.4	2.7	11	16	20	13	12	16	12	17	14.5	1.0	25.0	0.5054
09-Dec-91	**+4**	19	13	102	188	37	37	19	93	63.4	20.1	12	14	14	14	17	12	13	14	13.6	0.5	5.5	0.0030
16-Dec-91	**+5**	64	91	101	119	46	64	82	18	73.1	10.6	14	11	12	19	10	14	13	19	13.9	1.2	2.0	0.0006
23-Dec-91	**+6**	97	110	141	90	35	97	43	37	81.0	12.8	13	10	10	20	12	14	11	16	13.1	1.1	-	-
30-Dec-91	**+7**	137	110	37	165	46	64	37	64	82.2	16.1	14	12	11	12	13	13	11	15	12.5	0.4	-	-
06-Jan-92	**+8**	128	219	37	118	82	37	37	48	88.1	21.4	15	12	10	13	12	11	13	11	12.1	0.5	-	-
13-Jan-92	**+9**	128	219	9	37	18	46	183	55	86.7	26.3	13	9	10	16	10	12	16	10	11.9	0.9	-	-
20-Jan-92	**+10**	128	210	46	46	9	37	80	48	75.3	21.4	10	9	10	14	13	13	11	13	11.6	0.6	8.0	0.0104
27-Jan-92	**+11**	173	127	100	57	118	27	69	67	92.6	15.4	13	14	9	15	19	14	13	15	13.9	0.9	-	-
03-Feb-92	**+12**	137	37	192	37	110	46	110	55	90.1	18.6	13	13	16	12	16	13	17	18	14.8	0.7	-	-
10-Feb-92	**+13**	87	27	46	37	27	18	156	73	58.9	15.2	7	7	20	18	16	27	22	26	18.0	2.5	-	-
17-Feb-92	**+14**	91	87	73	49	73	38	118	67	74.5	8.3	14	13	14	19	13	11	18	15	14.5	0.9	-	-
24-Feb-92	**+15**	83	91	64	27	79	48	106	27	65.6	9.7	13	17	19	12	17	14	13	13	14.9	0.9	0.0	0.0002
02-Mar-92	**+16**	82	37	37	73	27	46	37	73	51.3	7.0	18	18	16	11	16	11	14	19	15.6	1.1	-	-
09-Mar-92	**+17**	174	74	27	49	19	18	29	18	51.1	17.6	18	21	17	17	17	17	15	20	17.8	0.6	-	-
16-Mar-92	**+18**	58	82	179	106	47	52	18	22	70.6	17.3	15	15	17	18	20	17	19	19	17.5	0.7	-	-
23-Mar-92	**+19**	146	146	46	62	69	44	29	22	70.5	16.3	18	23	17	14	15	22	12	9	16.2	1.6	-	-
30-Mar-92	**+20**	75	37	71	39	27	44	35	27	44.4	6.1	14	11	18	11	13	11	17	18	14.1	1.1	0.0	0.0002
06-Apr-92	**+21**	103	55	46	27	46	155	55	39	65.7	14.1	14	17	18	14	19	19	13	19	16.9	0.9	-	-
13-Apr-92	**+22**	30	64	56	23	47	55	36	22	41.5	5.4	15	15	18	15	13	16	20	16	15.8	0.7	-	-
20-Apr-92	**+23**	29	44	41	26	29	18	22	22	28.8	3.0	16	13	16	20	9	22	18	17	16.4	1.3	-	-
27-Apr-92	**+24**	22	36	64	37	82	64	36	27	46.0	7.0	18	13	9	11	11	9	18	18	13.5	1.4	-	-
04-May-92	**+25**	82	119	46	69	55	31	49	84	66.8	9.2	19	17	13	16	20	16	21	9	16.5	1.3	0.0	0.0002
11-May-92	**+26**	93	31	46	80	82	35	55	40	57.7	8.0	17	18	15	4	20	16	14	13	14.5	1.7	-	-
18-May-92	**+27**	24	93	58	44	64	9	60	44	49.5	8.5	14	12	13	16	16	14	16	16	14.4	0.5	-	-
25-May-92	**+28**	66	80	86	22	69	24	97	68	64.0	9.1	19	14	17	14	16	15	19	9	15.5	1.1	-	-
01-Jun-92	**+29**	38	18	81	64	15	27	22	28	36.7	7.8	20	16	15	11	14	13	18	11	14.5	1.0	-	-
08-Jun-92	**+30**	66	18	84	44	22	89	22	29	46.7	9.6	15	24	8	18	13	19	19	14	16.2	1.6	5.0	0.0003
15-Jun-92	**+31**	18	29	39	57	55	15	13	57	35.2	6.3	19	13	12	20	18	21	16	17	17.1	1.1	-	-
22-Jun-92	**+32**	68	78	27	26	40	83	13	27	45.3	9.0	16	14	16	17	14	15	20	18	16.0	0.7	-	-
29-Jun-92	**+33**	37	106	46	40	37	18	38	31	44.1	8.7	16	21	19	21	24	14	19	16	18.6	1.1	-	-
06-Jul-92	**+34**	20	44	69	78	24	20	68	60	47.9	8.0	18	14	11	18	24	12	18	22	16.9	1.5	-	-
13-Jul-92	**+35**	27	35	27	35	37	15	27	22	28.1	2.5	13	17	20	14	17	11	15	17	15.4	1.0	4.0	0.0019

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; weeks printed in bold faces indicate the period when the median values of the two groups were significantly different.

Appendix Table 8.5.19: Serum Gamma Glutamyl Transpeptidase Activity (U/l) Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
Date	Week	61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	U	P
07-Oct-91	-5	20	15	16	17	16	24	21	15	18.0	1.1	14	14	12	19	22	22	23	23	18.8	1.5	31.0	0.9591
14-Oct-91	-4	18	25	14	16	20	22	20	13	18.4	1.4	20	16	18	12	20	17	20	20	17.8	0.9	-	-
21-Oct-91	-3	24	29	20	17	15	25	29	17	22.0	1.8	16	13	12	21	28	19	19	22	18.6	1.7	-	-
28-Oct-91	-2	20	18	16	16	18	23	21	13	18.1	1.0	14	15	12	19	20	17	19	20	16.9	0.9	-	-
04-Nov-91	-1	22	25	16	16	25	30	27	15	22.1	1.9	16	16	10	16	29	24	23	19	19.2	1.9	-	-
11-Nov-91	0	24	18	13	13	22	27	20	13	18.7	1.9	14	14	12	14	25	21	21	22	17.9	1.6	31.5	0.9591
18-Nov-91	-1	20	25	14	16	25	25	21	16	20.4	1.5	22	25	18	16	21	17	19	20	19.8	1.0	-	-
25-Nov-91	-2	21	15	15	14	23	26	22	14	18.7	1.6	12	15	10	14	20	21	20	31	17.8	2.2	-	-
02-Dec-91	+3	25	23	15	14	23	22	21	15	19.8	1.5	13	14	13	12	23	23	16	29	17.7	2.1	-	-
09-Dec-91	-4	28	22	15	24	16	28	22	19	21.8	1.6	10	16	12	19	20	27	27	21	18.8	2.0	-	-
16-Dec-91	-5	32	35	23	17	23	25	23	23	25.3	1.8	16	25	23	29	29	25	17	29	24.1	1.7	31.5	0.9591
23-Dec-91	-6	42	32	23	22	27	27	27	23	27.8	2.1	17	17	29	17	21	23	17	33	21.9	2.0	-	-
30-Dec-91	-7	46	46	29	29	29	25	35	20	32.4	3.2	20	29	17	23	17	25	26	32	23.8	1.8	-	-
06-Jan-92	-8	69	29	17	17	17	30	23	17	27.6	5.9	16	22	16	19	20	21	20	23	19.5	0.8	-	-
13-Jan-92	-9	52	46	12	12	28	49	29	23	31.3	5.3	17	17	29	25	17	25	29	23	22.9	1.6	-	-
20-Jan-92	-10	64	40	23	23	23	98	23	23	39.8	9.2	23	23	17	12	25	25	29	15	21.1	1.9	20.0	0.2345
27-Jan-92	**+11**	110	75	46	29	29	110	35	75	63.7	11.3	23	29	25	29	19	17	19	14	21.8	1.8	2.0	0.0006
03-Feb-92	**+12**	127	127	52	98	35	104	41	52	79.6	12.9	17	21	16	23	16	22	15	17	18.5	1.0	0.0	0.0002
10-Feb-92	**+13**	110	133	81	133	29	104	35	87	89.0	13.2	14	13	14	17	13	25	6	25	15.9	2.2	0.0	0.0002
17-Feb-92	**+14**	32	106	71	46	35	175	11	153	78.7	19.9	22	16	29	27	16	23	23	23	22.6	1.5	8.0	0.0104
24-Feb-92	**+15**	87	81	58	41	59	133	23	81	70.3	11.1	21	13	16	23	23	17	16	11	17.4	1.5	1.0	0.0003
02-Mar-92	**+16**	87	69	75	75	41	145	64	87	80.3	9.9	18	17	23	16	16	17	17	17	17.7	0.8	-	-
09-Mar-92	**+17**	156	41	75	29	64	266	41	75	93.4	26.6	17	17	17	16	17	16	12	16	16.1	0.7	-	-
16-Mar-92	**+18**	139	149	70	98	98	93	46	98	98.9	11.0	12	46	12	17	46	12	6	17	21.0	5.3	-	-
23-Mar-92	**+19**	143	58	174	139	41	64	41	96	94.3	17.1	19	25	19	17	35	28	16	16	21.9	2.2	-	-
30-Mar-92	**+20**	98	81	98	64	64	17	162	84	83.6	13.6	14	17	25	25	12	25	10	19	18.5	2.0	3.5	0.0011
06-Apr-92	**+21**	151	98	41	46	46	75	64	40	70.0	12.7	18	16	17	20	22	22	15	16	18.2	0.9	-	-
13-Apr-92	**+22**	58	93	86	84	93	64	75	52	75.6	5.3	23	17	23	16	13	29	6	17	18.0	2.3	-	-
20-Apr-92	**+23**	156	35	72	75	41	46	35	41	62.5	13.6	12	23	23	16	29	23	17	14	19.7	1.9	-	-
27-Apr-92	**+24**	62	87	41	64	46	58	58	52	58.4	4.6	12	17	12	17	22	16	23	12	16.3	1.5	-	-
04-May-92	**+25**	75	35	64	86	12	116	75	96	69.7	11.0	19	12	29	19	23	17	13	17	18.7	1.8	7.6	0.0070
11-May-92	**+26**	17	52	23	110	12	123	35	249	77.6	26.8	12	16	30	19	12	16	13	13	16.1	2.0	-	-
18-May-92	**+27**	23	97	75	97	78	82	17	168	79.7	15.6	10	23	17	14	12	16	9	14	14.5	1.5	-	-
25-May-92	**+28**	84	81	139	69	58	112	17	17	72.3	14.0	12	28	9	16	11	12	12	22	15.2	2.2	-	-
01-Jun-92	**+29**	43	72	62	53	52	34	32	45	49.0	4.6	21	19	13	12	12	25	29	19	18.6	2.1	-	-
08-Jun-92	**+30**	41	61	56	26	17	27	58	63	43.6	6.0	9	13	12	10	16	16	19	26	15.0	1.8	2.5	0.0006
15-Jun-92	**+31**	34	110	37	75	29	56	58	58	57.0	8.7	13	30	13	12	21	17	21	26	19.1	2.2	-	-
22-Jun-92	**+32**	42	82	60	73	54	76	29	29	55.7	6.9	23	17	27	17	31	15	14	25	21.1	2.1	-	-
29-Jun-92	**+33**	43	67	37	42	51	40	27	28	42.0	4.3	16	14	14	16	13	15	14	15	14.5	0.4	-	-
06-Jul-92	**+34**	76	63	41	46	65	51	29	25	49.6	5.9	28	16	30	16	25	12	12	17	19.6	2.4	-	-
13-Jul-92	**+35**	51	49	51	46	41	42	26	32	42.3	3.0	13	15	18	12	23	14	17	12	15.5	1.2	0.0	0.0002

Statistics =results of Mann-Whitney U test; U =U statistic; P =two-tailed P value (probability); - =analysis not done; weeks printed in bold faces indicate the period when the median values of the two groups were significantly different.

Appendix Table 8.5.20: Serum Mineral Concentrations of Buffaloes
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)										Uninfected Animals (Group B)										Statistics	
		61	62	63	64	65	66	67	68	Mean	SE	71	72	73	74	75	76	77	78	Mean	SE	t	P
CALCIUM (mmol/l)																							
14-Oct-91	-4	1.47	1.46	2.08	1.83	1.61	1.36	1.58	1.72	1.64	0.08	1.75	1.63	1.68	1.52	2.28	2.04	1.74	1.83	1.81	0.08	1.50	0.1552
11-Nov-91	0	1.95	1.60	3.59	1.08	1.68	2.66	3.92	2.85	2.42	0.33	3.00	1.77	2.76	2.91	1.99	1.82	2.80	2.04	2.39	0.17	0.08	0.9367
09-Dec-91	+4	2.84	2.43	2.13	1.64	2.75	1.99	2.08	2.03	2.24	0.13	3.23	1.61	2.08	1.99	2.06	2.12	2.89	1.97	2.24	0.18	-	-
06-Jan-92	+8	2.58	2.50	2.38	1.98	2.61	2.17	1.97	2.38	2.32	0.08	1.80	3.32	2.16	1.50	2.84	2.30	3.07	2.79	2.47	0.21	0.67	0.5153
03-Feb-92	+12	2.58	2.49	2.63	2.56	2.83	2.15	2.05	2.21	2.44	0.09	2.81	2.53	2.07	3.15	2.71	2.47	2.43	2.32	2.56	0.11	0.84	0.4127
02-Mar-92	+16	2.28	2.77	1.90	1.89	2.72	3.13	2.96	1.94	2.45	0.17	2.42	2.42	2.41	2.56	2.34	2.71	2.34	2.10	2.41	0.06	0.22	0.8276
30-Mar-92	+20	2.15	2.29	2.30	2.15	2.35	2.15	2.26	2.55	2.28	0.04	2.32	2.32	2.42	2.47	2.13	2.20	2.13	2.10	2.26	0.05	0.31	0.7594
27-Apr-92	**+24**	2.15	2.01	2.24	2.14	2.13	2.27	2.17	2.46	2.20	0.04	2.33	2.64	2.59	2.64	2.35	2.48	2.46	2.28	2.47	0.05	4.22	0.0009
25-May-92	+28	2.35	2.45	2.31	1.88	2.18	2.61	2.11	2.23	2.27	0.07	2.36	2.19	2.20	2.82	2.38	2.38	2.48	2.24	2.38	0.07	1.11	0.2852
22-Jun-92	+32	2.45	1.94	2.26	2.30	2.17	2.06	2.13	2.58	2.24	0.07	2.41	2.32	2.31	2.34	2.39	2.11	2.77	2.50	2.39	0.06	1.63	0.1264
13-Jul-92	+35	2.34	2.11	1.99	2.27	2.16	2.17	2.24	2.44	2.22	0.05	2.41	2.22	2.27	2.38	2.56	2.34	2.23	2.15	2.32	0.04	1.56	0.1407
POTASSIUM (mmol/l)																							
14-Oct-91	-4	4.21	4.85	6.13	4.21	4.85	4.85	5.49	5.49	5.01	0.22	5.49	5.49	5.49	4.85	4.21	3.57	4.85	5.49	4.93	0.24	0.25	0.8095
11-Nov-91	0	4.85	4.85	4.85	5.44	4.65	5.44	5.49	6.13	5.24	0.16	4.47	5.49	4.21	6.13	4.85	4.85	5.49	6.13	5.20	0.24	0.14	0.8917
09-Dec-91	+4	3.96	4.85	2.93	4.85	6.13	6.77	5.49	5.49	5.06	0.40	4.95	4.95	4.78	5.49	5.49	4.85	4.85	6.13	5.19	0.16	0.30	0.7673
06-Jan-92	+8	4.85	5.36	5.13	5.11	5.11	5.62	6.31	5.62	5.39	0.15	5.49	4.85	5.36	5.87	5.36	4.85	4.85	6.30	5.37	0.17	0.09	0.9310
03-Feb-92	+12	4.85	5.62	5.11	4.85	4.60	5.36	4.85	4.85	5.01	0.11	4.85	6.13	5.11	5.11	5.36	4.85	5.62	5.85	5.36	0.16	1.80	0.0930
02-Mar-92	+16	5.49	4.85	6.13	6.13	5.49	4.85	6.13	5.49	5.57	0.18	6.77	6.77	4.85	5.49	4.85	5.49	6.13	6.13	5.81	0.25	0.78	0.4489
30-Mar-92	+20	4.85	4.21	4.85	4.21	4.85	5.49	6.13	4.85	4.93	0.21	4.85	5.49	4.85	5.49	4.85	4.85	4.85	4.85	5.01	0.10	0.34	0.7360
27-Apr-92	+24	4.85	4.21	4.85	4.85	5.40	4.21	3.57	4.85	4.60	0.19	5.49	4.85	4.21	4.85	4.85	4.85	4.85	4.85	4.85	0.11	1.14	0.2739
25-May-92	+28	3.45	4.21	4.85	4.85	4.21	4.85	5.49	3.57	4.44	0.23	4.21	4.57	4.21	4.57	4.57	4.57	4.57	4.85	4.52	0.07	0.33	0.7442
22-Jun-92	+32	6.13	5.75	5.36	4.85	4.85	5.49	6.77	5.87	5.63	0.21	5.49	5.23	4.60	5.11	4.60	5.62	5.23	6.13	5.25	0.17	1.41	0.1814
13-Jul-92	+35	5.85	6.13	5.36	5.62	5.75	5.23	6.31	4.98	5.65	0.15	5.49	5.87	5.23	4.60	5.23	5.49	5.36	6.00	5.41	0.14	1.17	0.2617
SODIUM (mmol/l)																							
14-Oct-91	-4	96	109	106	93	96	78	98	80	94.5	3.58	96	102	102	92	102	106	93	113	100.9	2.30	1.50	0.1548
11-Nov-91	0	104	78	111	93	92	80	104	98	95.2	3.82	90	106	117	106	91	100	102	113	103.3	3.18	1.63	0.1255
09-Dec-91	+4	117	111	78	117	109	112	106	87	104.7	4.75	124	106	117	91	122	111	104	117	111.6	3.55	1.16	0.2640
06-Jan-92	+8	115	122	124	96	102	117	124	104	112.9	3.59	85	113	126	115	124	117	112	126	114.8	4.42	0.33	0.7436
03-Feb-92	+12	113	122	126	128	120	124	126	139	124.7	2.49	128	128	139	139	140	124	117	134	131.2	2.74	1.76	0.1010
02-Mar-92	+16	111	141	109	115	117	122	133	113	120.0	3.76	126	128	117	122	113	113	106	113	117.3	2.46	0.60	0.5575
30-Mar-92	+20	106	104	109	111	117	96	122	122	110.8	2.99	126	102	96	102	126	96	96	106	106.3	4.28	0.86	0.4033
27-Apr-92	+24	96	115	112	99	117	111	117	75	105.3	4.86	109	106	96	102	109	133	100	102	107.0	3.72	0.28	0.7853
25-May-92	+28	78	109	122	99	113	113	104	124	107.8	4.78	96	103	109	92	104	92	96	106	99.8	2.17	1.52	0.1498
22-Jun-92	+32	96	117	96	104	120	109	109	113	107.8	2.96	113	122	117	104	120	106	117	111	113.8	2.06	1.66	0.1184
13-Jul-92	+35	104	106	96	96	116	104	104	112	104.9	2.37	106	122	113	103	128	103	117	115	113.4	3.00	2.46	0.0276
MAGNESIUM (mmol/l)																							
14-Oct-91	-4	0.30	0.44	1.26	0.81	0.99	0.83	0.68	0.49	0.73	0.10	0.58	0.78	0.72	0.74	0.72	0.69	0.68	0.70	0.70	0.02	0.29	0.7729
11-Nov-91	0	0.60	0.57	1.04	0.84	0.62	0.35	0.88	0.44	0.67	0.08	1.02	1.04	0.97	0.73	0.67	0.58	0.80	0.74	0.82	0.06	1.50	0.1558
09-Dec-91	+4	1.06	1.06	0.98	0.97	0.98	1.15	1.03	1.26	1.06	0.03	1.18	1.23	1.06	1.11	1.07	0.96	1.03	0.99	1.08	0.03	0.47	0.6446
06-Jan-92	+8	0.69	0.33	0.39	0.57	0.90	0.83	1.02	0.91	0.71	0.08	0.66	0.63	0.52	0.83	0.92	0.87	0.59	0.91	0.74	0.05	0.32	0.7552
03-Feb-92	**+12**	0.48	0.61	0.63	0.79	0.72	0.85	0.82	0.66	0.70	0.04	0.81	0.88	0.93	1.04	1.16	0.83	1.24	0.92	0.98	0.05	4.37	0.0006
02-Mar-92	**+16**	0.66	0.75	0.76	0.88	1.14	0.58	0.69	0.90	0.80	0.06	0.87	1.12	1.33	0.95	0.98	1.25	1.12	1.04	1.08	0.05	3.59	0.0030
30-Mar-92	+20	0.67	0.75	0.99	0.97	0.96	0.97	0.97	0.98	0.91	0.04	0.69	0.87	0.95	0.95	0.96	0.85	1.09	0.76	0.89	0.04	0.35	0.7289
27-Apr-92	+24	0.76	0.63	0.64	0.85	0.87	0.61	0.77	0.70	0.73	0.03	0.59	0.94	0.85	0.96	0.50	1.60	0.50	1.07	0.88	0.12	1.21	0.2453
25-May-92	+28	0.71	0.69	0.69	0.67	1.00	0.80	0.69	0.67	0.74	0.04	0.55	0.81	0.79	0.75	0.79	0.67	0.73	0.99	0.76	0.04	0.35	0.7289
22-Jun-92	+32	0.37	0.14	0.48	1.15	0.47	0.41	0.90	0.72	0.58	0.11	0.91	0.81	0.60	0.77	0.67	0.76	0.63	0.71	0.73	0.03	1.32	0.2095
13-Jul-92	+35	0.65	1.14	0.75	0.69	0.67	0.71	0.53	1.36	0.81	0.09	1.81	0.27	0.97	0.74	1.14	1.28	1.24	1.00	1.06	0.15	1.43	0.1749
INORGANIC PHOSPHORUS (mmol/l)																							
14-Oct-91	-4	1.42	1.28	0.90	1.20	1.03	1.29	1.24	1.74	1.26	0.08	1.29	1.25	1.38	1.31	1.23	1.42	1.13	1.17	1.27	0.03	0.12	0.9085
11-Nov-91	0	1.37	1.27	1.03	1.28	1.65	1.18	1.28	1.79	1.36	0.08	1.25	1.16	1.32	1.38	1.21	1.63	1.89	1.23	1.38	0.08	0.18	0.8622
09-Dec-91	+4	1.27	1.33	2.32	1.41	1.25	1.16	1.22	0.71	1.33	0.15	1.15	1.10	1.36	1.58	1.13	1.58	1.35	1.80	1.38	0.08	0.62	0.5428
06-Jan-92	+8	2.13	2.05	1.77	1.36	1.18	1.62	1.70	2.17	1.75	0.12	1.41	1.56	2.07	1.46	1.48	1.41	1.87	2.03	1.66	0.09	0.60	0.5581
03-Feb-92	+12	1.80	1.44	2.24	1.71	2.04	2.00	1.90	1.80	1.87	0.08	2.20	2.37	1.72	1.85	1.74	1.77	1.84	1.92	1.93	0.08	0.53	0.6042
02-Mar-92	**+16**	1.53	1.35	1.20	1.37	1.29	1.79	1.67	1.86	1.51	0.08	1.68	1.57	1.60	1.66	2.26	1.83	2.53	1.52	1.83	0.12	2.22	0.0435
30-Mar-92	+20	1.41	1.52	1.17	1.39	1.40	1.47	1.62	1.72	1.46	0.05	1.48	1.91	1.51	1.36	1.26	1.33	1.60	2.37	1.60	0.12	1.08	0.2997
27-Apr-92	+24	1.45	1.10	1.41	1.52	1.52	1.54	1.22	1.14	1.36	0.06	2.73	1.81	1.30	1.38	1.40	1.24	1.24	1.12	1.53	0.17	0.94	0.3617
25-May-92	+28	1.48	1.58	1.77	1.16	1.40	1.38	1.69	1.75	1.53	0.07	1.85	1.65	1.16	1.32	1.50	1.85	2.85	2.15	1.79	0.18	1.35	0.1996
22-Jun-92	+32	1.54	1.80	1.43	1.87	2.04	1.49	1.95	2.26	1.80	0.10	1.74	1.73	1.42	1.99	1.89	1.77	1.71	2.03	1.79	0.06	0.09	0.9329
13-Jul-92	+35	1.63	2.04	1.17	1.14	1.24	0.84	1.48	1.60	1.39	0.12	0.85	1.89	1.32	1.32	2.24	1.46	1.71	1.14	1.49	0.15	0.52	0.6108
COPPER (µmol/l)																							
14-Oct-91	-4	18.0	25.5	26.2	9.8	12.7	15.1	3.8	22.7	16.7	2.62	17.0	13.9	11.0	9.5	14.6	16.1	11.4	7.9	12.7	1.07	1.41	0.1794
11-Nov-91	0	13.0	20.1	27.9	20.2	20.5	17.6	10.4	26.0	19.5	1.95	9.2	11.0	21.9	13.0	14.7	20.2	10.1	28.0	16.0	2.21	1.19	0.2548
09-Dec-91	-4	19.1	12.9	12.7	14.0	13.6	15.8	14.7	16.0	14.8	0.70	10.8	11.0	15.0	13.8	13.6	20.6	28.8	25.6	17.4	2.25	1.10	0.2885
06-Jan-92	-8	10.1	10.1	8.7	12.7	13.3	10.8	10.8	22.0	12.3	1.38	15.8	10.0	11.9	10.8	14.6	22.6	21.8	23.6	16.4	1.83	1.79	0.0953
03-Feb-92	+12	8.0	12.6	9.6	10.0	10.2	13.2	12.1	20.4	12.0	1.26	11.1	10.4	28.0	22.8	11.6	23.8	10.4	14.8	16.6	2.36	1.71	0.1097
02-Mar-92	+16	9.3	12.7	8.0	10.4	8.0	7.2	14.0	20.0	11.2	1.41	24.0	11.2	17.2	12.0	10.4	11.2	11.2	18.8	14.5	1.64	1.53	0.1493
30-Mar-92	+20	11.2	15.6	11.6	9.4	12.9	16.4	12.8	23.6	14.2	1.47	22.8	8.8	17.0	12.2	15.2	20.0	19.6	10.4	15.8	1.65	0.72	0.4810
27-Apr-92	**+24**	11.2	12.0	15.2	12.2	15.2	15.2	17.2	24.8	15.4	1.43	29.2	28.8	26.8	21.6	16.0	16.0	23.6	18.0	22.5	1.81	3.08	0.0082
25-May-92	**+28**	17.7	13.9	14.0	11.2	14.2	15.2	22.7	27.8	17.1	1.83	22.3	23.5	20.7	26.1	26.7	24.1	16.5	23.2	22.9	1.07	2.74	0.0161
22-Jun-92	**+32**	13.7	13.7	13.7	13.1	10.2	14.0	14.9	14.9	13.5	0.49	14.9	11.4	18.4	27.9	26.6	26.6	17.7	11.4	19.4	2.26	2.55	0.0231
13-Jul-92	**+35**	11.7	10.2	18.6	9.7	8.0	11.1	12.0	10.2	11.4	1.04	15.7	18.8	18.0	17.1	24.0	18.0	17.1	17.0	18.2	0.83	6.38	0.0001

Statistics =results of unpaired t-test; t =t value; P =two-tailed P value (probability); the weeks when the means of infected and uninfected groupswere significantly different are printed in bold faces.

Appendix Table 8.5.21
Results of Agar Gel Diffusion Assay
Experiment 5: Infection of buffaloes with 400 *Fasciola gigantica* metacercariae

		Infected Animals (Group A)								Uninfected Animals (Group B)							
Date	Week	61	62	63	64	65	66	67	68	71	72	73	74	75	76	77	78
07-Oct-91	-5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14-Oct-91	-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21-Oct-91	-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28-Oct-91	-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04-Nov-91	-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11-Nov-91	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18-Nov-91	+1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25-Nov-91	+2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
02-Dec-91	+3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
09-Dec-91	+4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16-Dec-91	+5	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
23-Dec-91	+6	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
30-Dec-91	+7	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-
06-Jan-92	+8	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
13-Jan-92	+9	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
20-Jan-92	+10	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
27-Jan-92	+11	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
03-Feb-92	+12	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
10-Feb-92	+13	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
17-Feb-92	+14	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
24-Feb-92	+15	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
02-Mar-92	+16	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
09-Mar-92	+17	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-
16-Mar-92	+18	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
23-Mar-92	+19	-	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-
30-Mar-92	+20	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
06-Apr-92	+21	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-
13-Apr-92	+22	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-
20-Apr-92	+23	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-
27-Apr-92	+24	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
04-May-92	+25	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
11-May-92	+26	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
18-May-92	+27	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
25-May-92	+28	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
01-Jun-92	+29	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-
08-Jun-92	+30	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
15-Jun-92	+31	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
22-Jun-92	+32	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
29-Jun-92	+33	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
06-Jul-92	+34	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
13-Jul-92	+35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = positive for precipitin line; - = negative for precipitin line; ** =animals dead.